Behavioural and chemical ecology of *Meligethes aeneus*: effects of non-host plant volatiles

Thesis

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BEHAVIOURAL AND CHEMICAL ECOLOGY OF *MELIGETHES AENEUS*: EFFECTS OF NON-HOST PLANT VOLATILES

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ABSTRACT

The research in this thesis aims to develop an understanding of the olfactory aspects of host-location behaviour in the pest beetle *Meligethes aeneus* Fab., and to investigate the use of non-host plant volatiles as 'repellents' within a push-pull system of pest control for oilseed rape, which is being developed at Rothamsted Research.

Novel laboratory bioassays were developed and used to establish that essential oils from non-host plants reduce *M. aeneus* colonisation of oilseed rape flowers. Lavender essential oil had the greatest negative effect on beetle numbers on rapeseed flowers. This was further examined using a 4-arm olfactometer, and it was established that lavender oil alone elicits avoidance behaviours in *M. aeneus*, in addition to overriding their attraction to host-plant volatiles. The chemical basis of this effect was investigated using gas chromatography linked with electroantennography and mass spectrometry and the results are discussed in relation to the ecology of the insect.

It was established with semi-field studies that lavender essential oil reduces landing of *M. aeneus*, but does not affect the post-alighting behavioural process of host acceptance. These results are discussed in terms of behavioural plasticity in the importance of olfactory cues for host location such that, on alighting on a plant, the beetle may switch behavioural modes or may require a different suite of olfactory cues during host-acceptance behaviour. Field studies showed that lavender oil is effective at reducing natural movements of *M. aeneus* into plots of oilseed rape. Critically, the main reduction in infestation occurred during the vulnerable green-bud stage and the implications for optimal timing of repellent application during immigration are discussed. Flight patterns were further investigated using a novel combination of vertical-looking radar, suction traps and field counts to identify important meteorological and ecological factors.

The thesis presents an experimental progression in scale from the laboratory, to semi-field and field scale, for research into the effect of semiochemicals. The application and efficacy of using lavender or other non-host plant odours within a push-pull system of pest management for *M. aeneus* in oilseed rape are discussed.

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CHAPTER 1. GENERAL INTRODUCTION

This chapter reviews the host-location behaviour of phytophagous insects, and more specifically, the pollen beetle *Meligethes aeneus* Fab. (Coleoptera: Nitidulidae), focusing on the olfactory aspects of host-plant recognition by this species. Rejection or avoidance of non-host plants is integral to this behaviour and is evaluated in this thesis as an element of an integrated pest management strategy for the protection of oilseed rape, *Brassica napus* L. (Brassicaceae), against phytophagous insects. The importance of *M. aeneus* as a pest of oilseed rape is also discussed, along with current and potential strategies for its control.

1.1 OILSEED RAPE

Oilseed rape is a general term for all oilseed crops in the genus *Brassica* in the family *Brassicaceae*. The most dominant oilseed rape crop is swede rape (*Brassica napus* L.) which is a hybrid between *B. oleracea* L. (wild cabbage) and *B. rapa* L. (turnip rape). The earliest record of a *Brassica* grown for its seed is from the Netherlands in 1578. The crop has since spread through the rest of Europe, including Britain (Bunting, 1986). The initial use of the crop was for lamp oil, lubrication and cattle feed, but from the 1950s the proportion grown for vegetable oil has steadily increased. During the 1970s, oilseed rape started to be grown on a much larger scale in Britain (Alford *et al.*, 1991). This was mainly due to an EC policy of subsidies for oilseeds produced in Europe, making it economically viable (Winfield, 1992).

Winter and spring rape crops are grown. Winter rape is sown in August, flowers in May and is harvested in July. Spring rape is sown in March, flowers in June and is harvested in September. After flowering, the crop is left in the field for pod maturation before harvest.

Erucic acid and glucosinolates occur naturally in brassicas, but are unpalatable to humans. Therefore oilseed rape for human consumption and animal feed has been selectively bred for low levels of both compounds, leading to the 'double low' varieties now grown. *Brassica napus* contains over 30 different glucosinolates (Chen & Andreasson, 2001). These are sulphur-containing compounds that are important in defence against phytophagous insects (section 1.4.6.5). The plant can catabolise the glucosinolates when under insect attack and releases volatile isothiocyanates (section 1.4.6.5). These volatiles

are used for host location by many insects specialising on Brassicas (Bartlet *et al.*, 1993; Blight *et al.*, 1995a).

Since oilseed rape is grown for seed, it is one of only a few *Brassica* crops allowed to flower. The flowers are a bright yellow colour with four petals in the shape of a cross. Modern varieties of oilseed rape are almost completely self-fertilising so can yield well without insect pollination. However, the flowers are very attractive to many insects, because of their bright yellow colour and nectar content, and cross-pollination speeds up pod ripening, advancing the harvest.

1.1.1 Pests of oilseed rape

Pests of oilseed rape include generalist herbivores such as pigeons, slugs, rabbits, nematodes and insects, mostly comprising those adapted to the secondary metabolism of the *Brassica* genus. As already described, oilseed rape is attractive to many insects which attack all parts of the plant (Kirk, 1992). Despite being subject to pest attack, oilseed rape has an ability to compensate for insect damage, allowing up to 60% removal of pods without any yield loss (Williams & Free, 1979). Table 1.1 lists important insect pests of oilseed rape, all of which show some degree of adaptation to *Brassicas*, and the amount and type of damage they cause to both spring and winter oilseed rape.

1.1.1.1 Insect pests of oilseed rape

Table 1.1 Economically important insect pests of oilseed rape. (Taken from (Free & Williams, 1979; Alford *et al.*, 1991; Kirk, 1992; Winfield, 1992)). Asterisks represent low, moderate and high damage caused to spring and winter rape.

Name	Larval damage	Adult damage	Winter rape	Spring rape
Cabbage root fly (*Deila radicum* L.)	Roots		*	*
Cabbage leaf miners (*Phytomyza rufipes* Meigen)	Leaf petioles		*	*
Brassica pod midge (*Dasineura brassicae* Winnertz)	Seeds in pods		*	
Pollen beetles (*Meligethes aeneus* Fab.)	Pollen	Buds and pollen	**	***
Cabbage stem flea beetle (*Psylliodes chrysocephala* L.)	Petioles, stems and shoots		**	
Rape winter stem weevil (*Ceutorhynchus pictaris* Gyllenhall)	Leaf petioles, stems and plant crowns		**	
Cabbage seed weevil (*Ceutorhynchus assimilis* Paykull)	Seeds in pods	Developing pods	***	***
Cabbage stem weevil (*Ceutorhynchus quadridens* Panzer)	Leaf veins, stalks and main stems			*
Peach potato aphid (*Myzus persicae* Sulzer)	Leaves	Leaves and virus vector	*	*
Cabbage aphid (*Brevicoryne brassicae* L.)	Leaves	Leaves	*	*

1.1.1.2 Pollen beetles

Pollen or blossom beetles belong to several genera in the family Nitidulidae. The adults and larvae feed on pollen from many plant species, from which they obtain protein, amino acids, lipids, sterols, starch, vitamins and minerals (Roulston & Cane, 2000). *Meligethes aeneus* Fabricus is in the sub-family Meligethinae. They are black, oval beetles ranging in length from 1.9-2.7 mm. It has 11-segmented antennae with a compact 3-segmented club (Kirk-Spriggs, 1996). It is the most common and widespread species of pollen beetle in the UK, occurring in many habitats including woodlands, sand-dunes, coastal cliffs, waste ground, cultivated land and field margins (Kirk-Spriggs, 1996).

The adults overwinter in soil or under leaf litter and emerge in the spring and fly to food plants when the temperature exceeds 12°C. Gregarious migrations have been noted at temperatures above 13.5°C (Sedivy & Kocourek, 1994). The adults feed on pollen from many plant families, but oviposit exclusively on Brassicas. Each female can lay more than 200 eggs over a 2-month period (Hopkins & Ekbom, 1996), laying 1-3 eggs per bud, which hatch after 4-7 days. The larvae move up the oilseed rape raceme, feeding on pollen from flowers as they open. After feeding for 9-13 days, larvae drop to the soil surface and pupate for 14-18 days.

Table 1.2 details the timing of the different life stages of *M. aeneus* alongside the development of the oilseed rape crops. This highlights the fact that pollen beetles are serious pests of spring rape, due to their short life cycle in the spring, which leads to mass emergence of second-generation adults in June. Once oilseed rape stops flowering in July/August, *M. aeneus* adults move to other flowering plants to feed before overwintering diapause.

Table 1.2 Oilseed rape growing patterns and life cycle of *Meligethes aeneus*. (Taken from Williams & Free, 1978).

Time of year	Winter rape	Spring rape	Pollen beetles *Meligethes aeneus*
Aug/early Sept	Seeds sown		
Overwinter			Adults – under soil surface, in vegetation or leaf litter. Emerge >12°C. Feed on pollen of many plant families.
March		Seeds sown	
April – early	Green buds		Immigration to winter oilseed rape >12°C.
April – mid			Adults feeding in winter oilseed rape.
April – late	First flowers open		Maximum numbers in winter oilseed rape.
May – early			Mating and oviposition in winter oilseed rape.
May – mid	Full flower		Larvae developing on winter oilseed rape.
May – late		Green buds	Overwintered adults – migration from winter oilseed rape to spring oilseed rape.
June – early			High numbers of overwintered adults in spring oilseed rape.
June – mid	End of flowering Pods maturing	First flowers open	Larvae from winter oilseed rape pupating. Larvae developing on spring oilseed rape.
June – late			New generation adults (pupated in winter oilseed rape) move to spring oilseed rape.
July – early		End of flowering	Larvae from spring oilseed rape pupating.
July – mid	Harvest		New generation adults (pupated in spring oilseed rape) feed on spring oilseed rape.
July – late			Move to nearby plants e.g. wild crucifers (feed & oviposit). New generation adults do not mate – just feed then move to overwintering sites.
August/September		Harvest	

1.1.2 Damage to oilseed rape crops by pollen beetles

Adult *M. aeneus* feed on pollen throughout the budding and flowering stages. As indicated in Table 1.1, *M. aeneus* is one of the most serious pests of oilseed rape, especially of spring-sown rape. Females cause the most damage to the plants as they chew oviposition holes in the base of developing buds (Ekbom & Borg, 1996). Pre-flowering attacks often result in the appearance of podless stalks due to the loss of buds (Williams & Free, 1979). However, Brassicas naturally abort up to 50-60% of their flowers and buds, therefore, insect damage can be compensated for by retaining those that would have been aborted (Lamb, 1989). It is thought that oilseed rape can withstand 6-8 *M. aeneus* per plant at the 'separated green bud stage' without yield loss, even though there is visible damage to the crop (Lerin, 1988). However, higher numbers of *M. aeneus* can lead to yield loss. Pollen beetles cause most damage to the oilseed rape crop when it is at the green bud stage, and it is at this time, according to Free & Williams (1979) that the beetle numbers are highly concentrated at the edges of the fields (although see Chapter 8). It has also been suggested that oilseed rape acts as a reservoir for pollen beetles that attack other crops, e.g. strawberries, later in the year (Winfield, 1986).

Combinations of *M. aeneus* with other insect pests can lead to serious yield reductions. For example, an infestation of both *M. aeneus* and *Ceutorhynchus napi* weevils led to 10-20% yield loss in oilseed rape due to the plants being unable to compensate for early damage to the main shoot, whereas infestations of either pest individually did not lead to yield loss (Lerin, 1988). Despite these estimates of yield reductions, it has proved difficult to estimate the economic cost of insect damage to oilseed rape crops.

Meligethes aeneus populations have increased in response to the availability of large amounts of a good quality host plant (Hokkanen, 2000). The pest population is now distinct from non-pest populations for two ecological traits; increased reproductive success and intraspecific competitive ability. This study demonstrated the operation of evolutionary mechanisms that qualify an insect to be elevated to pest status. It is an indication that *M. aeneus* has adapted rapidly (within 15 years) to enable it to efficiently colonise the new crop plant and establish itself as an important pest.

1.2 HOST PLANT RECOGNITION BY PHYTOPHAGOUS INSECTS

All phytophagous insects exhibit a degree of selectivity in the plants that they eat, it therefore follows that they have behavioural mechanisms that enable them to choose the correct host plant of the correct quality (Schoonhoven, 1968). This behaviour is a step-wise process (Bernays & Chapman, 1994) and once a potential host has been encountered, a decision is made on whether to feed or oviposit on it, or to reject it and continue the search for a suitable host (Courtney *et al.*, 1989). The sequence of broadly defined behaviours is: orientation, landing, and probing followed by feeding or oviposition. These can be grouped into two stages; host location (detection/location of host from a distance) and host acceptance (having alighted, confirmation that the plant is of the correct species and quality). A range of stimuli associated with host and non-host plants mediates the behaviours in both stages. The sensory systems of sight, smell and taste are all utilised in this process and one of the most important is smell (olfaction) – the detection of volatile chemical odours.

Chemicals have been categorised by Dethier (1960) in terms of the behavioural responses elicited in insects:

1. Attractant: a chemical that causes an insect to make oriented movements towards the source of the stimulus

2. Repellent: a chemical that causes an insect to make oriented movements away from the source

3. Feeding or oviposition stimulant: a chemical that elicits feeding or oviposition. 'Feeding stimulant' is synonymous with 'phagostimulant'

4. Deterrent: a chemical that inhibits behaviours, such as feeding or oviposition

The first two categories describe chemicals involved in host-location behaviour as they can influence orientation to the source whereas the 3rd and 4th are important in host acceptance after contact with the potential host. Volatile chemicals have been further classified under the term 'semiochemicals' into pheromones and allelochemicals. These terms are described further in Section 1.4.6.

However, throughout this thesis the term **repellent** refers to a volatile chemical that is *avoided* by insects. This definition does not imply any behavioural mechanism for the reduction in number of insects, or time spent in areas, where the volatile chemical is

present. The term repellent has been used in this way in various other insect studies using similar methodologies to those used for the experiments in this thesis, such as the 4-arm olfactometer (Glinwood & Pettersson, 2000a), laboratory-based area avoidance tests (Chander *et al.,* 2000) and insect landings in flight chambers (Hori, 1998).

1.2.1 Host plant location

Olfaction and/or vision mediate long-distance location of host plants, and there are many examples of insects being attracted to host-plant odour (Nottingham *et al.*, 1991; Bartlet *et al.*, 1993; Ruther & Thiemann, 1997). The detection of an attractant odour is followed by orientation and movement towards the source (odour-conditioned upwind anemotaxis). Due to air turbulence, an odour released from a point source appears at a fixed point downwind as a series of odour filaments within a 'plume' (David *et al.*, 1983). Insects do not follow an odour gradient, as there is no gradient to follow (Bernays & Chapman, 1994). Instead, insects move upwind on stimulation by attractive odour filaments and when odour is lost, they fly across wind. This mechanism increases the probability of locating further odour (Bell *et al.*, 1995). David *et al.* (1982) demonstrated that persistently flying directly upwind at the point of contact with an odour filament would lead an insect directly to an odour source but wind direction changes and the insect will fly out of the odour plume. Thus casting across wind and upwind anemotaxis on odour detection are critical to successful chemo-orientation by flying insects.

Host-plant odour is a combination of ubiquitous volatiles found in all green plants and some highly specific chemicals released by only a few plant species. An example of species-specific volatiles is the release of isothiocyanates by brassicas (section 1.4.6.5). These specific volatiles can act as a quick plant identification cue to insects. In addition to specific volatiles, all plants release a range of volatile chemicals as a result of oxidative degradation of leaf lipids. These include hexanol, hexanal and other compounds with 6-carbon chains that are collectively called 'green leaf volatiles'. The exact chemical composition of the green leaf volatile profile varies between plant species so insects are also able to detect and utilise these odour cues in host location. This is exemplified by the attraction of the Colorado beetle, *Leptinotarsa decemlinata* Say to the specific mixture of green leaf volatiles from potato plants (Visser & Ave, 1978). In addition, flowers emit a further profile of volatiles that is distinct from that emitted by the vegetative plant parts (Dobson, 1994).

The insects' attraction to their host-plant volatiles can be disrupted by changes in the relative proportions of chemicals within these volatile profiles. This indicates that more complex olfactory information, such as ratios and blends, is gathered rather than simply presence/absence of chemicals. Such quantitative assessments can be achieved by the use of paired olfactory cells such as those on the antennae of the cabbage seed weevil *Ceutorhynchus assmilis*, that enable the insect to determine the ratio of two specific chemicals in a blend (Blight *et al.*, 1995b).

1.2.1.1 Host location behaviour of M. aeneus

Meligethes aeneus are known to be attracted to both visual and olfactory stimuli associated with their host plants (Blight & Smart, 1999). Visual attraction to plants might result from responding to the colour or shape recognition of the host plant. However, these vary greatly even within a given plant species, so it is likely that visual responses only occur in the presence of an appropriate olfactory stimulus (Bernays & Chapman, 1994). Conversely, the response to an odour is also affected by the visual cues, for example, a field trapping experiment showed that the magnitude of the odour effect on trap catches of *M. aeneus* was dependent on the nature of the visual cue (Blight & Smart, 1999). Yellow was the most attractive trap colour to *M. aeneus*, followed by yellow/green and white, whereas cream, grass green and black were unattractive (Blight & Smart, 1999). Also, *M. aeneus* showed significant preference for yellow flowers, as opposed to cream or white flowers in varieties of oilseed rape, even though they had same odour profile (Giamoustaris & Mithen, 1996).

Meligethes aeneus is responsive to a large number of chemically diverse volatiles, which may be an adaptation that has led to its polyphagous nature (Smart & Blight, 2000). Volatiles from its main host plant, *B. napus*, are attractive (Free & Williams, 1978; Ruther & Thiemann, 1997; Blight & Smart, 1999; Smart & Blight, 2000) and were found to be significantly preferred over other known attractant plant volatiles (Ruther & Thiemann, 1997) and part of the attractive odour emanates from pollen (Cook *et al.*, 2002a). Intact plants in the early bud stage (i.e. when the crop is lacking the attractive yellow flower colour) can also elicit this attraction response, indicating that *M. aeneus* has the ability to locate host plants by olfactory stimuli (other than floral volatiles) alone (Ruther & Thiemann, 1997). Such attraction has been demonstrated in the field using baited traps, where *M. aeneus* was shown to have the ability to locate sources of rape odour over

distances of at least 20 m (Evans & Allen-Williams, 1994). No-one has observed the flights directly and such an attempt forms part of Chapter 6.

1.2.2 Host plant acceptance after landing

Having landed on a plant, insects determine if it is of the correct species and quality. Olfactory discrimination is still important at this stage and odours from the leaf are still present around the leaf surface. Since the colour of a plant varies greatly, it is likely that visual attraction only occurs with the correct olfactory signal. Other visual signals include the shape and size of the leaves and the wavelength and intensity of the reflected light. The physical properties of the leaf surface, such as texture, hairs and waxes are also important host-confirmation factors (Bernays & Chapman, 1994).

Host-confirmation factors gained immediately after landing on the plant are important in the selection of oviposition sites. For example, the turnip root fly *Delia floralis* accepts or rejects a plant for oviposition after landing and performing a sequence of behaviours (Hopkins *et al.*, 1996). Specific odours such as oviposition deterrent pheromones or odours from conspecific eggs give ovipositing females further information about the suitability of the host (Den Otter *et al.*, 1980).

Taste and contact chemosensors are the final tests of the plant before consumption (Schoonhoven, 1968). Indeed, detection of factors during stylet penetration of their host plant is sufficient to inhibit take-off and induce feeding in the aphid *Aphis fabae* (Powell & Hardie, 2000).

1.2.2.1 Host-acceptance behaviour of M. aeneus

Meligethes aeneus remain on host plants (Brassica species) after landing, whereas they only stay for a short time on less preferred plants (*Sinapis alba*) (Borg & Ekbom, 1996). However, this depends on the physiological state of the insect, and *M. aeneus* eventually accepted *S. alba* as an oviposition host after prolonged exposure to the plant in a no-choice test. *Meligethes aeneus* have a wider host range for feeding than for oviposition. Therefore it follows that females conduct different assessments of chemical and physical information in the behavioural patterns observed prior to feeding or oviposition (Ekbom & Borg, 1996). This is discussed in relation to the findings in Chapters 6 and 7.

1.2.3 Non-host plant recognition and avoidance

Non-host plants are those plants not used by an insect for oviposition or feeding and are recognised using similar cues as described in the previous section on host finding. Non-hosts are either avoided due to the lack of key host recognition factors or due to the presence of repellent or deterrent stimuli indicating, for example, that the plant is unsuitable due to antibiotic defences or inappropriate for the growth stage of the insect (Pickett *et al.*, 1995). All phytophagous insects investigated can detect components of general green leaf volatiles (Bernays & Chapman, 1994). Due to this general sensitivity, it is likely that all phytophagous insects can smell any plant and thereby make generalised olfactory distinctions between hosts and non-hosts. Detection of plant-species specific volatiles can improve this sensitivity to allow further discrimination. Host chemistry (both volatile and non-volatile) has been shown to be the most important factor in macroevolutionary patterns of host use, promoting host shifts in phytophagous insects (Becerra & Venable, 1999).

Insects often land upon non-host plants, but critical decisions are made on close inspection of the plant. The physical properties of the leaf surface are important in the detection of non-hosts, but consumption of plant material enables the insect to assess the balance between phagostimulants and deterrents which determines whether the insect will feed or not (Bernays & Chapman, 1994). Contact chemicals on the plant surface can also be important and can be detected by receptors on the tarsi, antennae or palps. Host quality is also likely to be assessed in this way.

Insects show plasticity in their acceptance or rejection of plants for feeding or oviposition (Bernays, 1999). The acceptance of a plant as a host depends on the condition of the insect, plant and the abiotic environment. For example, water-deprived insects often tolerate deterrents and so feed on non-host plants, especially the petioles where water content is highest. Also, female insects are often less selective in host-plant choice when carrying a large egg load due to their higher energy requirements.

1.2.3.1 Non-host plant recognition and avoidance in M. aeneus

Ruther & Theiemann (1997) studied the response of *M. aeneus* to volatiles from host and non-host plants in a Y-tube olfactometer, and concluded that there must be some specific compounds which enable the beetles to distinguish between their host plants and other

plants. They suggested that flowering oilseed rape emits several compounds, such as isothiocyanates, that were missing in the plants used in their study. However, the behavioural response of *M. aeneus* non-host plant odours during host location has not been studied. Therefore, a main aim of the work in this thesis is to characterise alterations in the behavioural patterns of *M. aeneus* during host location, in response to non-host plant odour.

1.3 OLFACTORY RECEPTION

Most insect olfactory organs, chemoreceptive sensillae, are on the antennae and are functionally adapted to respond to airborne volatiles (Visser, 1986). Odour molecules diffuse through pores in the sensillum cuticle to the dendrites of sensory cells. Odourant binding proteins solubilise the odour molecule and transfer it to receptors on the dendritic membranes. When bound this causes an initial depolarisation of the sensory cell membrane, known as a receptor potential. The magnitude of the receptor potential is graded according to the strength of the stimulus and once a depolarisation threshold is reached, action potentials are fired with a frequency related to the strength of the stimulus. The action potentials are transmitted to central nervous system where they are processed and translated into motor functions of the peripheral nervous system.

The olfactory neurones are either specialist; allowing discrimination of a limited array of stimuli and identifying volatiles of importance to the insect, or generalist; showing a response to a number of compounds (Field *et al.*, 2000). Pheromone receptors are specialised to respond to one or a few compounds. Most however are generalist, detecting a variety of food-odour components. The differential detection of odours by receptors enables an across-fibre pattern to code for odour quality (Dethier, 1982). In this way the insect can detect a large range of chemicals yet only respond behaviourally to the correct blend. Minimal concentrations for detection of pure odourants vary between $1.9x10^{9}$ and $4.3x10^{11}$ molecules/cm^3 (Smith & Getz, 1994) and the concentration of the odour is also important in determining the response of the insect. Due to their high sensitivity to odours, attractants can become repellent at very high concentrations. There are also species-specific deterrent cells, which respond to a range of secondary plant metabolites that inhibit feeding (van Loon, 1996). This behavioural avoidance response can be affected by mutations in a single gene encoding an ion channel, DSC1, showing that selectivity occurs throughout the process that leads to the resultant behaviour (Kulkarni *et al.*, 2002). Other

factors determining the behavioural response of an insect to a specific odour include genetic variability in the behavioural response (Campan *et al.*, 2002), the physiological state of the insect, presence of feeding stimulants or deterrents, learning and feeding experience (Dethier, 1982).

1.4 CONTROL OF *M. aeneus* IN OILSEED RAPE

As discussed in Section 1.1.2, *M. aeneus* can cause significant damage to oilseed rape crops. Therefore several control strategies are employed to reduce the pest populations within the crop.

1.4.1 Monitoring

There is a need for methods of accurate monitoring to help farmers establish the level of pest infestation and therefore assess the need for control. Action or economic thresholds are set for each species to limit the number of spray applications. ADAS recommendations are 15-20 pollen beetles per plant for winter rape, however this can be as low as 5 beetles per plant for very susceptible crops. For spring rape, the threshold is an average of 2-3 beetles per plant at any time from very early green bud to yellow bud (ADAS, 1984). In Denmark, the threshold is set as low as 1 beetle/plant in the early bud stage (Nielsen & Axelsen, 1988). These thresholds are based on data from field trials including knowledge of the pest's biology, damage to the crop, effects on yield and response to insecticides.

In order to adhere to the recommended thresholds, there is a need for efficient monitoring methods, since early detection of the pest is essential (ADAS, 1984; Nielsen & Axelsen, 1988). There are active and passive methods for monitoring pests. Active monitoring involves counting the number of insects present in sample transects across the whole crop. Nielsen & Axelsen (1988) provide a sampling method for pollen beetles. Passive monitoring involves the use of traps, such as water or sticky traps that can be unbaited or baited with attractants such as pheromones (Smart *et al.*, 1993; Sedivy & Kocourek, 1994). Knowledge of the biology of the pests can also help to predict where infestations are likely to occur (Finch *et al.*, 1990). For example, there are temperature thresholds below which the insects cannot fly (Kjaerpedersen, 1992; Sedivy & Kocourek, 1994).

1.4.2 Chemical control

The most common pest control method for oilseed rape is the spraying of synthetic insecticides. Broad-spectrum insecticides are used in the UK, currently approved pesticides for pollen beetle control in oilseed rape are mainly pyrethroids, including alpha-cypermethrin, deltamethrin, zeta-cypermethrin amongst others (Whitehead, 2000). Pesticide applications are the cheapest and most effective method of pest control, as they only cost £2-£3 per hectare. However, pyrethroids have a broad spectrum of activity, making them dangerous to bees and other beneficials. This means that selectivity needs to be introduced in terms of management by timing or method of formulation and application (Graham-Bryce, 1987). Only spraying at times and in places where the pest outbreaks are or might occur, means that insecticides are used more efficiently. Also, the type of insecticide can provide further specificity to the target pest species. In both winter and spring sown oilseed rape, adult *M. aeneus* are more numerous at the edges of a field compared to the centre (Free & Williams, 1979; Nielsen & Axelsen, 1988). This is more pronounced at the early stage of invasion (April/May for winter sown rape and early June for spring rape). So, spray applications could be specifically targeted at the field edges during the critical stage of infestation to control the population before it reaches the peak.

1.4.2.1 Problems with insecticides

Insecticides kill pest species, but they may also kill many beneficial and non-target organisms present within the crop (Vickerman, 1992). There are specific guidelines on the responsible use of pesticides in order to protect honey bees and wild bees due to their important role as pollinators of crops (Whitehead, 2000). Also, the loss of natural enemies of the pest species within the crop means there is less predation of the pests which often results in the need for multiple insecticide applications per crop (Burn, 1992). Due to this type of pest management causing extreme selection pressure on the pests, populations of *M. aeneus* in Scandinavia have developed resistance to pesticides (Ekbom & Kuusk, 2001; Hansen, 2001). Sub-lethal effects of insecticides can also affect the behaviour of non-targets insects, as well as accumulating in the food chain causing knock-on effects on farmland birds and mammals (Carson, 1963). Leaching and run-off of insecticides into watercourses and their persistence in the soil can also cause environmental damage. Such environmental considerations over the problems associated with the use of insecticides have led to the development of alternative control strategies. The rest of this section details the types of approaches that are appropriate for control of insect pests in oilseed rape.

1.4.3 Integrated pest management

Integrated pest management (IPM) requires use of insecticides only in response to pest numbers exceeding economic thresholds. Understanding of the spatial pattern of insects following colonisation can help reduce pesticide inputs and prevent killing beneficials such as parasitoids (Murchie *et al.*, 1999). Cultural strategies such as crop rotation and the enhancement of biological control are used to reduce the need for insecticide intervention. Crop rotation is a traditional method used to reduce the build up of pests and diseases, and it is recommended that oilseed rape is grown in rotation with cereal crops and should not be grown more than once in five years (Lockhart *et al.*, 1993). Such traditional control methods are still important, but do not reduce pests below the economic thresholds. Therefore, IPM also includes enhancing populations of naturally-occurring biological control agents, use of resistant cultivars and manipulation of pests using behaviour-modifying compounds (Evans & Scarisbrick, 1994).

1.4.4 Biological control

Biological control involves the use of natural enemies to suppress pests population densities. The three main methods for the use of natural enemies are conservation, introduction and augmentation (Van Driesche & Bellows, 1996). For oilseed rape pests, various strategies have been investigated. For example, pathogenic fungi such as *Metarhizium anisopliae* (Butt *et al.*, 1998) and *Beauveria bassiana* (Hokkanen, 1993) have been used to infect adult pollen beetles and achieved significant mortality. Manipulation and enhancement of natural enemy populations can help to control pests in arable crops. In oilseed rape, carabids and parasitoids have been shown to be strongly spatially associated with their prey (Ferguson *et al.*, 2000; Warner *et al.*, 2000). This association can be manipulated, for example by using synthetic insect pheromones, to co-ordinate the maximal influx of beneficials into the crop during pest outbreaks, providing an environmentally benign method of control. Additionally, increased structural complexity of the landscape surrounding oilseed rape enhances parasitoid populations and results in increased parasitism of pollen beetle larvae in the field (Thies & Tscharntke, 1999).

1.4.5 Crop cultivars

Variations in plant susceptibility to pests arise naturally or can be manipulated for IPM (Van Emden, 1987). In addition to plant breeding, oilseed rape has been genetically engineered with *Bt* protein genes for resistance to lepidopteran pests (Stewart Jr *et al.*,

1996). Similarly, genetic manipulation of the crop can be used to reduce the release of plant specific volatiles used by pest species in host-location (Bartlet *et al.*, 1999b).

1.4.6 Semiochemicals in pest control

Central to many methods of IPM is the use of plant- or insect-derived semiochemicals or 'signal chemicals'. These are chemical odours that are important in communication between organisms and so can be manipulated for behavioural disruption of the pests. It has been 40 years since the first insect pheromone was discovered, yet semiochemical based products still only constitute less than 1% of the world pest control market (Jones, 1998). This is possibly due to under-investment in the technology (Hillman, 1998), as well as the fact that insecticides are often cheaper, can be applied more easily and do not require the users' understanding of the ecological interactions. However, with an increasing number of insecticides being banned from use due to their negative environmental impacts, semiochemical-based products should provide an alternative.

Semiochemicals can be divided into pheromones and allelochemicals. Pheromones are chemicals which, when released, influence the behaviour or development of other individuals of the same species, whereas an allelochemical functions in interspecific communication. However, the same chemical can be classed in both categories as many natural enemies have evolved to use pheromones emitted by their prey species as a means of locating them, e.g. aphid parasitoids are attracted to the aphid sex pheromone (Hardie *et al.*, 1991). Semiochemicals can be used in pest control as insect attractants, repellents or deterrents.

1.4.6.1 Insect pheromone attractants

Attractant pheromones can be used to bait traps for monitoring pest populations, as well as in control by attracting insects to specific areas that can subsequently be treated with insecticides. Several insect attractant pheromones have been identified, which include sex and aggregation pheromones. For example, the aphid sex pheromone has been characterised for several species and it has been found to comprise two main compounds - nepetalactone and nepetalactol in varying ratios depending on the aphid species (Dawson *et al.*, 1987). Aggregation pheromones have been identified for flea beetles *Phyllotreta cruciferea* (Goeze) and unmated female *Ceutorhynchus assimilis* weevils (Evans & Bergeron, 1994).

1.4.6.2 Insect pheromone repellents

Repellent insect pheromones can be used to reduce pest colonisation of the main crop area by inducing emigration from the treated areas. The aphid alarm pheromone (mainly composed of the sesquiterpene hydrocarbon (*E*)-β–farnesene) has been used successfully to increase aphid mobility within the crop to increase the pick-up of pathogens or pesticides (Griffiths & Pickett, 1987). An epideictic (or spacing) pheromone has been identified in *M. aeneus* which has the effect of preventing other conspecifics from infesting the same host plant (Ruther & Thiemann, 1997).

Ovipositing female *Ceutorhynchus assimilis* are known to deposit an oviposition deterring pheromone after egg-laying to deter other females from laying in the same pods (Ferguson & Williams, 1989). However, these pheromones are volatile and so have short-lived effects. Characterisation of these chemicals could lead to the synthesis of artificial compounds in a slow-release formulation that could be sprayed on the crop to act as repellents.

1.4.6.3 Allelochemicals

Allelochemicals, chemicals released by an individual that elicit specific responses in individuals of a different species, can be further described in terms of the benefits to the emitter and receiver. Allomones benefit the producer by the effect it invokes in the receiver. Kairomones benefit the receiver but are disadvantageous to the producer whereas synomones benefit both the receiver and the producer. All types of allelochemical signals can be utilised in pest control strategies.

1.4.6.4 Insect-derived allelochemicals

Chemicals released by insects can be found in the pheromones of many different species (Leal *et al.*, 1994) or are highly species specific. These chemicals can be detected by generalist or specialist predatory or parasitic species, which use them as attractant cues to locate their prey/hosts. In this case, the compound is acting as a kairomone. For example, carabids, coccinelid beetles and parasitoids are attracted by the alarm and sex pheromones of aphids and use this as an efficient means of locating their prey (Powell, 1998; Al Abassi *et al.*, 2000).

1.4.6.5 Plant-derived allelochemicals

Plant volatiles are used as kairomones by phytophagous insects to locate their host plants (section 1.1). Despite this, plants still benefit from the release of volatile chemicals as the natural enemies of phytophagous insects use the volatiles as an indirect method for locating their prey, and therefore in this interaction the volatiles are acting as synomones.

Plants also release volatile synomone signals that aim to reduce attack from phytophagous insects, for example, by repelling insects (Bernasconi *et al.*, 1998) or reducing oviposition (Kessler & Baldwin, 2001). For example, *B. napus* stores glucosinolates (naturally occurring secondary plant chemicals) in cell vacuoles. On tissue damage, the glucosinolates are brought into contact with the enzyme myrosinase (which is compartmentalised elsewhere in the plant) and are hydrolysed into a wide range of degredation products (Chen & Andreasson, 2001). Glucosinolates and their catabolites, particularly isothiocyanates, provide the first chemical barrier to deter a broad spectrum of herbivores and pathogens. Isothiocyanates are volatiles and act as synomones, signalling to phytophagous insects that the plant contains toxins and therefore are repellent to most insects. These volatiles are characteristic of the Brassica family and some phytophagous insects have evolved to become Brassica specialists, by developing physiological ways to overcome the toxins and have adapted to use the isothiocyanates as kairomones to recognise their host plants (Blight *et al.*, 1992; Bartlet *et al.*, 1993).

The antennae of pollen beetles have been shown to detect 25 oilseed rape volatiles, including isothiocyanates (Blight *et al.*, 1995a; Blight *et al.*, 1995b). The most active are 2-phenylethyl, 3-butenyl and 4-pentenyl isothiocyanates and all are attractive in the field to both *M. aeneus* (Smart *et al.*, 1995) and *C. assimlis* (Blight *et al.*, 1995a). These compounds also stimulate feeding and oviposition. But this attraction is concentration dependent, as they can act as weak repellents at sufficiently high concentrations; even to adapted oilseed rape pests (Dawson *et al.*, 1993).

The volatiles produced by plants when damaged, can act as a 'distress signal' attracting parasitoids and stimulating their foraging behaviour. In this instance, such a signal is known as a synomone. Bradburne & Mithen (2000) showed that the enhanced production of the 3-butenyl isothiocyanate in oilseed rape increases the attraction of the aphid parasitoid, *Diaeretiella rapae*. Therefore, on releasing isothiocyanates the plant repels non-

brassicaceous insects while attracting both specialised pests and their parasitoids. However this situation is a snapshot within the evolutionary arms race and all three trophic levels are continuously evolving to utilise the situation further to their advantage.

1.4.7 Push-Pull Strategy or Stimulo-Deterrent Diversionary Strategy

The idea of using several elements in a formalised strategy originated from work from two control programmes. The combination of deeply planted onion culls (trap crops) and chemical oviposition deterrents on onion seedlings succeeded in greatly reducing oviposition by the onion fly *Delia antiqua* on the seedlings (Miller & Cowles, 1990). This bi-polar manipulation was found to be far better than either component alone and it was termed stimulo-deterrent diversion (SDD). At the same time, Pyke *et al.* (1987) termed this control concept the push-pull strategy in relation to work on control of *Heliothis spp.* From these beginnings, several other push-pull strategies have been developed for other pests including mountain pine beetles, pea and bean weevils (Smart *et al.*, 1994), German cockroaches (Nalyanya *et al.*, 2000) and maize stem borers (Khan *et al.*, 2000).

A combination of attractant and repellent semiochemicals can be used to manipulate pest and natural enemy populations (Pickett *et al.*, 1997; Agelopoulos *et al.*, 1999). Figure 1.1 represents the theory schematically. Within the push-pull system both pests and natural enemies are manipulated using semiochemicals. The pests are 'pushed' out of the crop using plant-derived antifeedants, oviposition-deterring pheromones, repellent crop cultivars and repellent non-host plants that disrupt the host-location behaviour of the pests. The remaining pests are controlled in the crop by attracting natural enemies. The pests are also 'pulled' away from the crop into trap crops using attractants such as sex pheromones. The trap crop is composed of plants which are designed to be even more attractive to the pest insect than the crop to be harvested. The trap crop can also be treated with a selective insecticide or a pathogen.

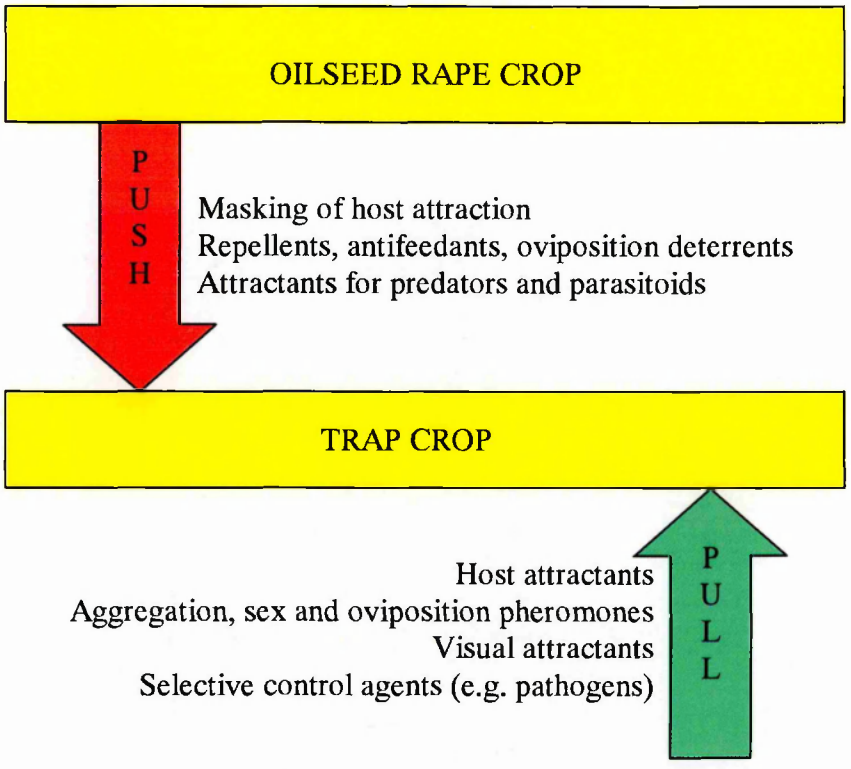


Figure 1.1 The push-pull or stimulo-deterrent diversionary strategy

Each component of the system is relatively ineffective compared to the use of broad-spectrum insecticides, but used together they have an additive or even synergistic effect (Jones, 1998). The combination of relatively weak elements has the added advantage of not selecting strongly for resistance in the pest species (Pickett, 1998). In order to develop a push-pull strategy, the effects of all the individual components need to be researched, to find compatible semiochemicals (Smart *et al.*, 1997b).

An example of a UK push-pull strategy is the development of control methods for the pea and bean weevil, *Sitona lineatus* L. Field trials demonstrated that *S. lineatus* populations could be aggregated by pheromone application and deterred with neem oil as an antifeedant (Smart *et al.*, 1994). This work is complimentary to a parallel semiochemical strategy in peas, using aphid sex pheromone to attract parasitoids to control pea aphids (Smart *et al.*, 1997b). These systems are compatible, whereas the use of a broad-spectrum insecticide to control the weevils on the pea crop would affect the parasitoid populations thereby reducing their effectiveness for aphid control.

In order to design a push-pull strategy for oilseed rape insect pests, several semiochemical components need to be discovered. These include attractants and repellents for both pests and natural enemies, which then need to be field tested to ensure there are no unexpected side effects. It is essential that the host-location behaviour of the pests be fully understood, as it is mediated by complex communications between the pest and the host plant, often of a very specific chemical nature. This understanding will allow efficient deployment of the semiochemical strategy.

1.4.8 Applications for manipulating host-finding behaviour of *M. aeneus*

An approach to disrupting the host-finding behaviour of insect pests of oilseed rape is to manipulate the level of isothiocyanates and other volatiles released from the crop. The volatile profile of the plant can be manipulated in two ways.

Firstly, the main crop could be modified or bred to produce less of the volatiles, thereby reducing the kairomonal signal used in insect host-location. For example, interrupting the genes involved in the synthesis of the alkenyl glucosinolates, which give rise to the attractive isothiocyanates, may make the crop less attractive to pest insects (Evans, 1995). However, these low glucosinolate varieties may become more susceptible to generalist herbivores (Milford *et al.*, 1989) and disease attack and become less attractive to parasitoids and other natural enemies (Murchie *et al.*, 1995). It is therefore essential that all the theoretical components of the push-pull strategy are field-tested for their effects on all potential pests and beneficials.

Alternatively, plants could be identified or selectively bred to produce an increased volatile signal and used as an attractant trap crop. Trap crops are grown to attract insects and thereby afford protection to the target crop by preventing the pests reaching the crop or concentrating them in a certain part of the field where they can be destroyed (Hokkanen, 1991). Two main strategies of trap cropping have been developed. Firstly, the trap crop can consist of plants that are more attractive to the insect than the main crop plant. Indeed *M. aeneus* is now successfully controlled on cauliflower crops with a trap cropping system using strips planted with several species of trap plants, including chinese cabbage and calabrese (Hokkanen *et al.*, 1986). *Meligethes aeneus* is controlled in oilseed rape using an alternative strategy, which is to use a border of the main crop plant, timed to be at the most attractive stage at the critical time for pest control, when the main crop is not yet attractive

(Hokkanen, 1989). A combination of these approaches, using turnip rape (*Brassica rapa*) trap crop borders around oilseed rape fields, also reduces *M. aeneus* infestations along with several other pest species (Buechi, 1990; Cook *et al.*, 2002b). The reasons for its effectiveness are that it is a light-green colour and that it flowers earlier than oilseed rape, thereby attracting the ovipositing females away from the main crop (Buechi, 1990) where they can be controlled with insecticide before the main crop flowers.

Research is continuing to identify the most effective plants and crop cultivars for use as trap crops and is also establishing the most efficient system of planting. Trap cropping is normally used as part of an integrated pest control system and the effectiveness is enhanced by combination with other control measures such as repellent odours.

1.4.9 Potential for using non-host plants in pest control

The presence of non-host plants can make host location by phytophagous insects more difficult (Ramert & Ekbom, 1996). Non-host plants can be planted either as an intercrop or a border to reduce pest infestations. Root (1973) suggested two explanations for the lower levels of monophagous insect infestations in diverse habitats. 1) The enemies hypothesis; predators and parasites are more abundant and effective in diverse systems than in homogeneous ones, 2) The resource concentration hypothesis; monophagous insects are more likely to locate and remain in homogenous systems rather than diverse systems due to the higher availability of food (the same applies to *M. aeneus* as it only oviposits on brassicas making them more likely to remain in homogenous systems due to the higher availability of oviposition sites). However, as the following review of intercropping examples shows, there are often many mechanisms involved in the reduction of pest damage in polycultures.

1.4.9.1 Physical or visual disruption

Physical obstruction from the intercrop plants can reduce the colonisation rate of crops by impeding low-altitude flights (Coll & Bottrell, 1994). Diverse systems have associational resistance from higher taxonomic and microclimatic complexity, thereby reducing outbreaks of herbivores (Tahvanainen & Root, 1972). Additionally, non-host plants can reduce the chance of the insects landing on a host plant. Insects assess plant quality having landed on a potential host plant, and landing on a non-host plant during this process can cause the insect to leave the area. Another possible mechanism for such results is that

intercropping interferes with the insect's visual cues. The cabbage root fly infests fewer cabbages in the field when the cabbages are intercropped with clover; this reduction in pest numbers has been attributed to a confusion effect as the cabbages no longer stand out as green surrounded by brown soil (Finch & Collier, 2000). A similar mechanism (reduced host plant 'apparency') was proposed to explain the reduction in colonisation of onions by *Thrips tabaci* Lind. when mix-cropped with carrots (Uvah & Coaker, 1984)

1.4.9.2 Olfactory disruption

Currently, most uses of non-host plants in pest control are found in intercropping systems where the non-host plant alters the volatile profile around the host plant by masking the host odour. For example, intercropping maize plants with non-host molasses grass can control lepidopteran stem borers (Khan *et al.*, 1997; Khan *et al.*, 2000). Such intercropping significantly decreased the levels of infestation by stem-borers in the main crop and also increased larval parasitism of stem borers by a parasitoid. This was proposed to be mediated by the volatiles released from the intercrop grass, which repelled the foraging female stem borers, yet attracted the parasitoids.

Intercropping can lead to lower pest infestations, however the choice of companion or protector plant is important. For example, a reduction in lepidopteran pest infestations was achieved when broccoli was intercropped with yellow sweetclover, but the yield of broccoli was reduced compared to a monocrop, because the intercrop competed for light and space (Hooks & Johnson, 2001). Additionally, natural enemy interactions need to be considered, as parasitoids have been shown to respond to simple and diverse plant assemblages in a similar manner to herbivores, i.e. immigration was higher in monocultures and tenure time was shorter in intercropped habitats (Coll & Bottrell, 1996). Finally, the timing of sowing for the intercrops and main crops is important to encourage maximum populations of natural enemies within the system (Parajulee *et al.*, 1997). The use of intercropping as a pest control method, without the use of pesticides, has received increasing interest over the past decade and has an important application in organic farming (Theunissen, 1997).

1.4.9.3 Non-host plant extracts

The main aim of this thesis is to identify repellent non-host plant odours to the pest *M. aeneus* which can reduce the number of pests on the crop and therefore be used in pest

control. There have not been any repellents identified for this species and they form an integral part of the developing push-pull strategy. If a non-host plant produces volatiles that are repellent to pest insects, but is not effective or practical to use the whole plant in intercropping, extracts or essential oils can be utilised (Onesimus *et al.*, 1998). One way of extracting volatiles is to extract the essential oils from the plant. 'Essential oil' is a broad term covering any oily, volatile substances obtained from vegetable sources (Dethier, 1947). They are generally liquid at room temperature and volatilise without decomposition. Essential oils are obtained from every part of a plant, often several can be extracted from different parts of the same plant, including the flowers, seeds, leaves, buds, roots, fruits and sap. Essential oils can be extracted from the plant using one of several processes; expression, steam distillation, water distillation or extraction with volatile solvents (Williams, 1996). The oils are composed of a mixture of organic chemicals in varying proportions and with different volatilities. Therefore, throughout this thesis, individual batches of essential oils were used to provide a standard (i.e. consistent), volatile, non-host plant cue for use in behavioural testing.

In order to determine the composition of an essential oil, several analytical techniques can be used. The separation of the constituents can be achieved using gas chromatography (GC) (McNair & Bonelli, 1969) and the elucidation of molecular structure of each constituent requires mass spectrometry (MS) or infrared spectrophotometry. Williams (1996) provides a good overview of these techniques. Such techniques were employed in Chapter 5 to determine the chemical composition of the essential oils.

Many essential oils have known insect repellent and insecticidal properties and have been successfully used to control pests of stored products (Sarac & Tunc, 1995). The insecticidal properties of several essential oils have been demonstrated to act via contact, fumigant or residual toxicity mechanisms. Negative anemotaxis or repellency has been shown in many insects to many essential oils (Sarac & Tunc, 1995; Bekele *et al.*, 1996; Mumcuoglu *et al.*, 1996; Bekele *et al.*, 1997; Ngoh *et al.*, 1998; Landolt *et al.*, 1999). Theoretically, they can also be used to repel insect pests from crop plants due to their non-host volatile profile (Hori, 1998).

1.4.9.4 Inducible plant-plant defence systems

Attack from plant pathogens and herbivores can influence gene expression and induce plant defence systems (Pickett & Poppy, 2001). Herbivore-induced emissions of maize volatiles have been shown to repel aphids while also attracting natural enemies with the same emissions (Bernasconi *et al.*, 1998). Mechanical damage to the leaves does not induce these changes in gene expression. Methyl salicylate is released by tobacco plants inoculated with tobacco mosaic virus and acts as an airborne signal to activate disease resistance and the expression of defence-related genes in neighbouring plants (Shulaev *et al.*, 1997). There is also evidence that volatiles from undamaged plants can affect the leaf temperature and physiology of a neighbouring plant, causing a decrease in attraction to aphids (Pettersson *et al.*, 1999). In crop protection, switching on defence genes using benign chemical signals is a better strategy than engineering or plant breeding, which can cause strong selection for resistance in pests (Pickett & Poppy, 2001).

1.5 TECHNIQUES FOR STUDYING PHYSIOLOGICAL RESPONSES OF INSECTS TO ODOURS

There are several laboratory- and field-based techniques that can be employed to determine whether an insect can detect an odour. Following these, behavioural assays can establish whether the detection of the odour leads to a behavioural response (Hare, 1998). A logical sequence of experimental investigations into semiochemically-mediated behaviour is listed by Poppy (1991). He suggests the following order:

1. Behavioural observations
2. Chemical extraction
3. Electrophysiological investigation
4. Chemical identification/synthesis
5. Behavioural bioassays
6. Field trials.

Behavioural observations, such as the avoidance of a plant species by an insect, can be an indication of a semiochemically-mediated behaviour (Chapters 3 & 4). This can be investigated further by the extraction of the volatile or non-volatile chemicals from the avoided plants. Electrophysiological techniques can be used to determine whether the insects' peripheral receptors are able to detect that particular odour (Chapter 5). The electroantennogram (EAG) measures receptor potentials across an isolated antenna and the

amplitude of the response is proportional to the concentration of the chemical stimulus, until a saturation level is reached (Wadhams, 1991). Action potentials from single olfactory cells can be recorded using extracellular microelectrodes implanted at the base of sensillae or a 'surface-contact' technique (Den Otter *et al.*, 1980), which provides detailed information on the response from one or a few receptors.

Having established that the odour is detected by the insects' peripheral receptors, it is possible to identify the compounds within the complex odour. The components can be analysed using GC and MS, in the same way as essential oils. If many volatile compounds are present, GC can be coupled with the EAG to determine which of the volatiles are being detected (Wadhams *et al.*, 1994). Analogues of the most important compounds can be synthesised (Pickett & Woodcock, 1991) for testing in behavioural assays. Not all the components of the odour that are detectable by the insect elicit a behavioural response. Therefore, the next stage is to identify which of the compounds are biologically active. This can be done using the synthesised analogues, but, as already mentioned, using the correct ratio of the main components of an odour is important in eliciting the natural response from the insect.

The odour from plants or essential oils can be tested for biological activity using lab-based behavioural bioassays or with bait traps in the field to sample the responses of the natural populations (Chapters 6 & 7). However, the use of olfactometers (Chapter 4) enables description of an individual insect's response and behaviours when in contact with an odour. This allows comparative and qualitative assessment of behaviours.

1.6 OBJECTIVES

As already detailed, masking or repellent odours from non-host plants can influence the movement of insects (Section 1.4.9). A repellent non-host plant for use in an oilseed rape push-pull strategy is a non-Brassica that produces volatiles that are avoided by pest insects. This investigation was centred on identifying a repellent non-host plant, which acts to disrupt the host location behaviour of the pollen beetles *Meligethes aeneus*. This olfactory disruption was targeted at altering the beetles' host location and acceptance behaviour resulting in a reduction in colonisation of oilseed rape crop plants. An understanding of the alterations in the response of the insects to such odour was developed at expanding spatial and temporal scales.

CHAPTER 2. GENERAL METHODS

Methods that were used routinely throughout the experiments are described in this chapter. Methods that have been developed as part of this research, or that are specific to certain sections of the thesis, are described in the relevant chapters. All work described in this thesis was conducted between May 2000 and September 2002 at Rothamsted Research, Harpenden, Hertfordshire, U.K. (see Appendix 2 for farm map) Ordnance Survey TL1213.

2.1 OILSEED RAPE PLANTS

Spring oilseed rape *Brassica napus*, cultivar Aries, was planted weekly from the beginning of March to end of July each year, and grown in a glasshouse to produce flowering plants from May to August. The glasshouse conditions were maintained at 20 °C (day) and 10 °C (night) and plants were watered daily. Plants were grown individually from seed in 8" pots and they usually took 8 weeks from sowing to reach the flowering stage (growth stage 65 according to Lancashire's growth stage key, 1991, Table 2.3). Flowering racemes were cut from the plants and used to feed insect cultures (section 2.2) or individual flowers removed for use in the experiments in Chapters 3 & 4. Undamaged flowering plants (approx. 1.5 m tall) were used in Chapter 6 for the semi-field experiments (Figure 2.1).

Figure 2.1 Flowering potted plants of oilseed rape in semi-field cage

2.2 POLLEN BEETLES

2.2.1 Collection and culturing

Adult pollen beetles *Meligethes aeneus* (Figure 2.2) were collected by sweep netting in winter-sown and spring-sown oilseed rape fields throughout the flowering periods. The insects were identified, sexed (section 2.2.2) and then kept in ventilated, plastic sandwich boxes, dimensions 175 x 115 x 60 mm (Stewart Plastics, Surrey, UK) and fed on flowering racemes of glasshouse-grown oilseed rape (section 2.1). The cultures were maintained in a constant environment room at 10 °C with a 16:8 hours light:dark regime. The beetles were separated into single sex cultures (see section 2.2.2) and kept in culture for no longer than 4 days before being used in behaviour experiments.

Figure 2.2 *Meligethes aeneus* on an oilseed rape flower

Meligethes aeneus is difficult to rear in large numbers in laboratory cultures (Bromand, 1983). The insects used in the experiments throughout this research were all field-caught adults and therefore their age, physiological state, feeding and mating experiences were unknown.

2.2.2 Identification and sexing

Individuals of *M. aeneus* were separated from other pollen beetles using the key in Kirk-Spriggs (1996). When sexing pollen beetles, they were first chilled in glass pots on ice to reduce their movement. Individual beetles were removed from the pot using a paintbrush and placed on their dorsal surface on a glass slide. A glass coverslip was placed on the ventral surface of the beetle and gentle pressure exerted on the lower abdomen to extrude part of the genitalia. The genitalia were examined under a binocular dissecting microscope and compared to diagrams in the book by Kirk-Spriggs (1996).

2.3 ESSENTIAL OILS

The essential oils used in this research were obtained from commercial sources (Table 2.1) with the exceptions of the pineapple mayweed (*Chamomilla suaveolens* (Pursh) Rybd. Asteraceae) and gum haggar (African *Commiphora sp.* (Burseraceae)) extracts (see Chapter 4 for details). Table 2.1 shows the available details about the oils. They were all extracted by steam distillation.

Table 2.1 Essential oil sources

Essential oil	Plant species	Country of origin	Source
Eucalyptus	*Eucalyptus globulus*	China	Boots, UK
Lavender	*Lavandula angustifolia*	Bulgaria	Boots, UK
Ti tree	*Melaleuca alternifolia*	Australia	Boots, UK
Peppermint	*Mentha piperita*	USA	Boots, UK
Geranium	*Pelargonium graveolens*	China	Boots, UK
Lavender	*Lavandula angustifolia (v.* Mailette)	England	Botanix, UK

The sources were Botanix (Botanix Limited, Hop Pocket Lane, Paddock Wood, Kent, TN12 6DQ) and Boots (The Boots Company Plc, Nottingham, England. Botanics range of aromatheraphy products). The essential oils were stored at 5 °C and diluted in acetone to experimental concentrations when required. The essential oils from Boots were used in chapter 3, however, from chapter 4 onwards only the Botanix lavender oil was used. Ray Mariott from Botanix supplied lavender oil from the same extract.

2.4 ESSENTIAL OIL SACHETS

The non-host plant odours were dispensed from slow-release sachets containing sponges dosed with the essential oil (section 2.4.1). These sachets were used to release the odour of the essential oils at an almost constant rate in semi-field (chapter 6) and field (chapter 7) experiments.

2.4.1 Preparation of sachets

Sponges made from blue, cellulose sponge cloths (J. Sainsbury plc) of two thicknesses; 3 mm and 10 mm, were washed in warm soapy water and then thoroughly rinsed in hot water. The sponges were oven dried at 24 °C and cut into small rectangles (2.5 cm x 2 cm). Some colourants were removed by continuous extraction in chloroform using a Soxhlet extraction apparatus for 24 hours in order to remove as much of the blue dye as possible, however, not all the colour could be removed. The chloroform was evaporated from the sponges in a fume cupboard.

Polyethylene flattened tubing (5 cm wide) was used to contain the sponges (Figure 2.3). The following thicknesses, or gauge (G), were used; 100G, 250G, 500G and 1000G. 2000G was achieved by enclosing a 1000G sachet inside another piece of 1000G tubing (Figure 2.4). Polyethylene tubing was cut to length and sealed at one end using an impulse heat sealer machine. A sponge piece was placed inside the tubing and 0.3 ml of test compound was added to the sponge. The tubing was immediately sealed at the other end. A hole was punched in the polythene at the top to enable the sachet to be fixed to canes in the experiments (Figure 2.5). The sachets were stored at –20 °C until needed.

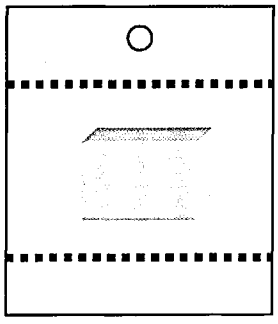


Figure 2.3 1000G sachet containing a sponge

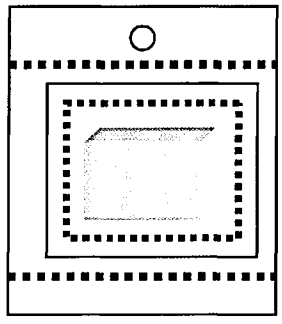


Figure 2.4 1000G bag containing a 1000G sachet to make a 2000G sachet

Figure 2.5 Sachet attached to a water trap in an oilseed rape field

2.4.2 Estimation of release rates from sachets

For each experiment, the required release rates were selected using the following method. Four sachets of each type listed in Table 2.2 were prepared (section 2.5) and lavender essential oil (Botanix) applied to each sponge. These sachet types were selected to provide a range of release rates. Immediately after sealing, the sachets were weighed and then hung in an airflow chamber set at 0.2 metres/second in a 20 °C constant temperature room. The sachets were weighed every 2-3 days over a two-week period.

For each sachet gauge, the mean accumulated weight loss was calculated and plotted over time. Figure 2.6 is an example of such a plot. A linear regression was fitted to each data set with the intercept set at 0 and the equations solved to calculate weight losses (release rate) per day (Table 2.2). Linear regression was used to allow direct comparisons amongst the sachets and, in general, the R^2 values show that this was a good fit to the data.

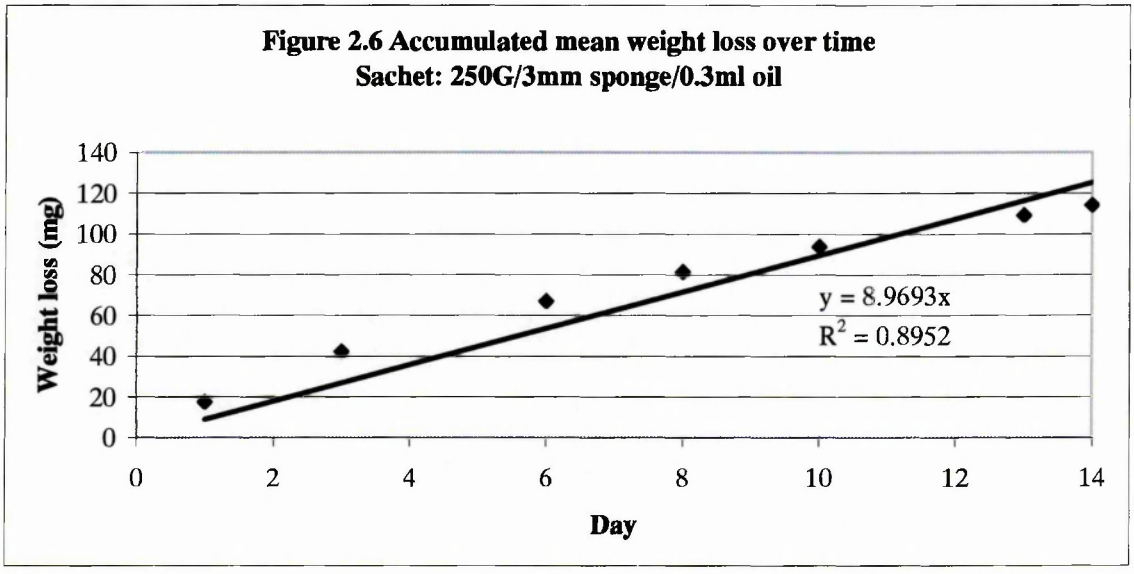


Table 2.2 Results of estimation of release rates for 10 sachet types

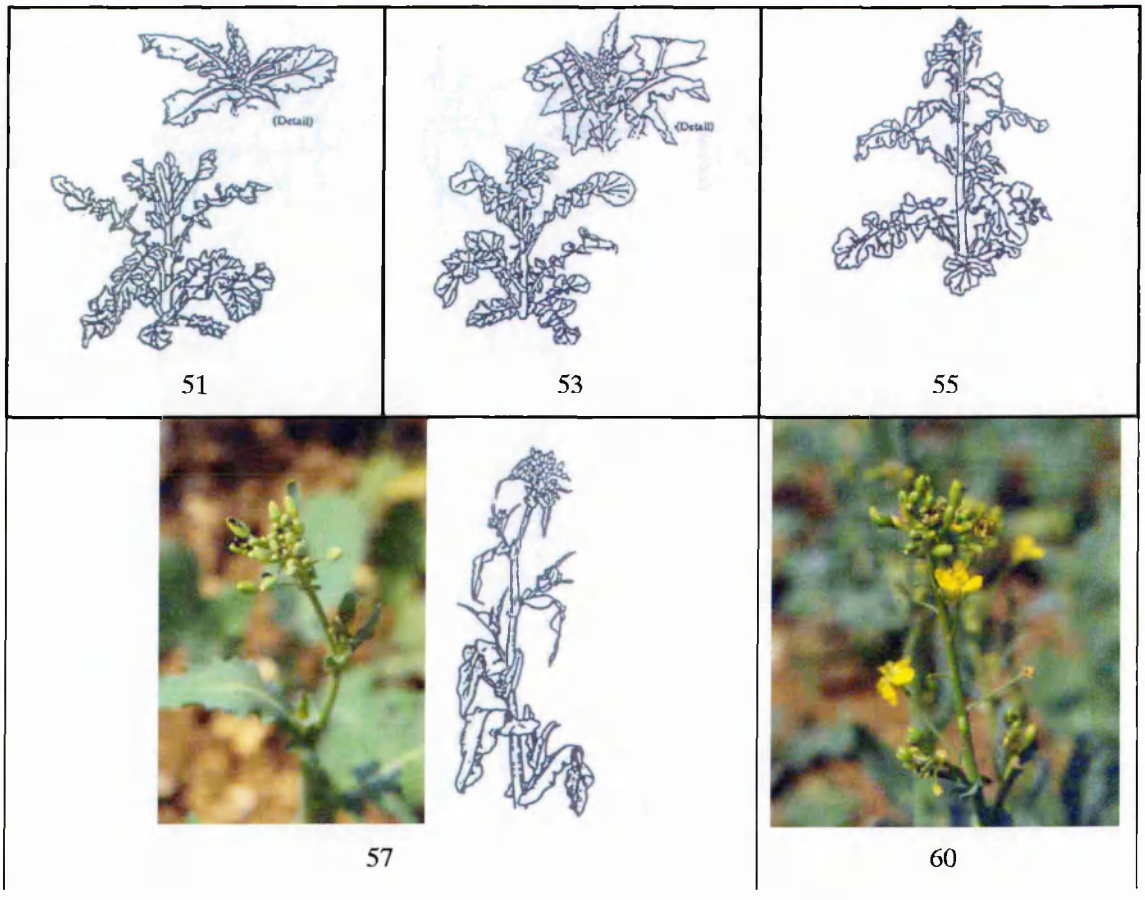
Gauge	Sponge thickness (mm)	Volume of oil (ml)	R^2	Release rate (mg/day)	Where used?
2000	3	0.3	0.993	1.1	Chapter 7
2000	3 (half sponge)	0.3	0.978	1.6	
2000	11	0.3	0.981	2.7	
1000	3	0.3	0.984	4.3	
500	3	0.3	0.952	7	
250	3	0.3	0.895	9	Chapters 6 & 7
250	11	0.3	0.947	10.3	
250	3	0.6	0.968	17	
250	3	0.8	0.964	18.5	
100	3	0.3	0.640	20.1	

2.5 GROWTH STAGE ASSESSMENT OF OILSEED RAPE PLANTS IN THE FIELD

The overall growth stage of field plants of oilseed rape was assessed for experiments in chapters 7 and 8 using the BBCH codes and descriptions given in Lancashire (1991). The plants were scored from the early stages of inflorescence emergence until the end of flowering, therefore only the inflorescence emergence and flowering codes were used. Those plants without any inflorescence were scored as zero.

Table 2.3 BBCH growth stage codes, descriptions and photographs. Adapted from Lancashire (1991).

Inflorescence emergence	
50	Flower buds present, still enclosed by leaves
51	Flower buds present visible from above ("green bud")
52	Flower buds free, level with the youngest leaves
53	Flower buds raised above the youngest leaves
55	Individual flower buds (main inflorescence) visible but still closed
57	Individual flower buds (secondary inflorescences) visible but still closed
59	First petals visible, flower buds still closed ("yellow bud")
Flowering	
60	First flowers open
61	10% of flowers on main raceme open, main raceme elongating
63	30% of flowers on main raceme open
65	Full flowering: 50% of flowers on main raceme open, older petals falling
67	Flowering declining: majority of petals fallen
69	End of flowering



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CHAPTER 3. THE EFFECT OF ESSENTIAL OILS ON HOST COLONISATION BY *MELIGETHES AENEUS* IN LABORATORY BIOASSAYS

3.1 INTRODUCTION

Plants have a suite of chemicals that protect them from phytophagous insects. There are two main ways for the plant to use them in defence, one is to release them as volatiles and the second is to store non-essential secondary metabolites in an inactive form or compartmentalised. The first plant-related chemical stimuli an insect might encounter are the volatiles released when the stomata are open. These volatile chemicals include a wide variety of short chain alcohols, aldehydes, ketones, esters, aromatic phenols and lactones, as well as mono- and sesquiterpenes (Bernays & Chapman, 1994). These are referred to as the green leaf volatiles. In addition to these green leaf volatiles, plants release specific chemicals, which often give a characteristic odour. The smaller molecular weight compounds are usually the most volatile and therefore of most importance in detection of plants from a distance. Close-range (within approximately 1 cm) inspection of a plant enables the insects to come into contact with a more complex mixture of volatiles which includes the higher molecular weight compounds.

The secondary metabolites of plants are biologically active compounds, which are often toxic to herbivores. Again, these chemicals vary between species and can contribute to their characteristic odour. These secondary compounds are often toxic to the plant as well and therefore are stored in specialised compartments or are combined with sugars, salts or proteins to produce innocuous compounds (Bernays & Chapman, 1994). An example of this is the storage of glucosinolates in brassica plants, as described in section 1.4.6.5, where upon damage to the tissue by insect feeding, the enzyme myrosinase comes into contact with the glucosinolates resulting in the release of a range of volatiles (Chen & Andreasson, 2001). These are mainly isothiocyanates that are released by the intact plant at low concentrations, but are released at much higher concentrations when the plant is damaged. The wound-induced volatile compounds are the second set of odours that host-seeking insects can detect at a distance.

Manipulation of the glucosinolate-myrosinase levels within crops can reduce herbivory by generalist insects however, it has generally failed to reduce herbivory in specialist insects

(Kliebenstein *et al.*, 2002). This is because brassica specialists, including *M. aeneus*, have evolved adaptations to enable them to feed on these plants without adverse effects, for example the mustard aphid *Lipahis erysimi* sequesters glucosinolates and retains them in the body (Dilawari *et al.*, 1997). Whereas many generalist phytophagous insects are unable to metabolise the glucosinolates and still use the volatile isothiocyanates as signals indicating toxic food plants (Ryan & Byrne, 1988; Kliebenstein *et al.*, 2002). These also often function as 'stress signals' and are used by foraging parasitoids and predators as synomones to enable them to locate their insect hosts (Bradburne & Mithen, 2000).

Meligethes aeneus is polyphagous on emergence from over-wintering and feeds on pollen from plants in a wide range of families (Free & Williams, 1978; Kirk-Spriggs, 1996). Oviposition only takes place on Brassicas, so a narrowing of host acceptance for females occurs on sexual maturity. Due to this large range of host plants, it follows that *M. aeneus* is attracted to a wide range of plant-derived volatiles. *M. aeneus* has been shown to respond to general green leaf volatiles from both host and non-host plants (Ruther & Thiemann, 1997) and also to volatiles characteristic of Brassicas, such as isothiocyanates (Smart & Blight, 2000).

Non-host plants are plants that are not used by the insect for feeding or oviposition at any stage of their lifecycle. Non-hosts include plants with repellent or toxic properties and, as discussed in section 1.2.3, non-host plants can be avoided in response to volatile cues detected from a distance. Identification of the most important volatiles and non-volatiles used by *M. aeneus* to avoid non-hosts would enable non-host odours to be incorporated into a control strategy to act as repellents.

For experimentation, a source of plant volatiles can be obtained by head-space entrainment or from steam or water distillation. Head-space entrainment collects the volatiles from around intact plants (Knudsen *et al.*, 1993). In order to collect secondary metabolites that are released on insect damage, the plants need to be infested with the pest prior to entrainment. In this chapter, essential oils were used to provide a standard, volatile, non-host plant cue for use in behavioural testing. Steam or water distillation enables extraction of all volatile components from a plant providing a wider range of volatiles for behavioural testing, which is advantageous. However, the essential oil extracted in this way may contain artefacts due to hydrolysis of volatile esters and extraction of non-volatile

constituents within the plant material. For example, linalool treated by steam distillation is degraded into several other products resulting in up to a 60% loss in content (Pickett *et al.*, 1975).

However, an advantage of using essential oils is that a large quantity of oil can be extracted and used as a consistent product, whereas head-space extraction produces variable volatile profiles only in small quantities. However, head-space extraction remains the best way to non-destructively sample the volatiles emitted from intact plants or parts of plants.

Laboratory bioassays investigating the behavioural response of phytophagous insects to plant volatiles are usually specific to the system in question. In general, investigation is centred on the modification of the behavioural response of the insect in the presence of plant volatiles compared to 'control' conditions. Examples of methodologies to investigate non-host plant odours include the use of olfactometers (see review in chapter 4), wind tunnels (Nottingham & Hardie, 1993; Evans & Allen-Williams, 1998) and application of plant extracts to food sources in laboratory and field arenas (Bekele *et al.*, 1996; Bekele *et al.*, 1997). Electrophysiological responses can also be used to identify antennal detection of plant odours (Nottingham *et al.*, 1991; Hardie *et al.*, 1994; Groot *et al.*, 1999) (Chapter 5).

However, the methodologies listed above require prior knowledge of the insects' behaviour in order to select the most appropriate method. Several studies have been conducted to characterise the response of *M. aeneus* to host-plant odours, but none to date have investigated their response to non-host plant odours. In order to compare several non-host plant odours, a simple laboratory assay is required to identify the modifications of behaviour in the presence of non-host plant odour. No-choice tests investigating preferences of one food source compared to another are limited in their interpretation as the situations offered are only between eating and starving (Dethier, 1982), therefore, a more rigorous test is to present two or more choices. A simple Petri-dish bioassay was used by Scheffler (1993) to study plant extracts for their repellency to the German cockroach *Blatella germanica* L. and the measure of behaviour was summarised as a Repellency Value. This basic preference testing method was modified for use in this study by introducing airflow to prevent saturation of the arena with volatiles, thereby presenting two distinct olfactory choices.

In this chapter the odours investigated were from a range of commercially available essential oils with known insecticidal or insect repellent properties (Sellar, 1992). These essential oils are all extracted from plants that do not appear on the list of host plants of *M. aeneus* (Kirk-Spriggs, 1996) and can be assumed to be non-hosts of the beetle. The non-host plant essential oils were tested at a range of concentrations for behavioural effects on *M. aeneus*. This is the first step in Poppy's (1991) scheme for testing semiochemically-mediated behaviour.

3.2 AIMS

1. To develop a novel laboratory-based method for testing the initial behavioural response of *M. aeneus* to a range of odours in controlled conditions.
2. To investigate the behavioural responses of *M. aeneus* to a range of non-host plant essential oils in controlled conditions.
3. To select one essential oil with strong repellent properties for further behavioural testing.

3.3 MATERIALS AND METHODS

3.3.1 Equipment

The bioassays were conducted in box arenas (Figure 3.1). Each box was 175 x 115 x 60 mm (Stewart Plastics, Surrey, UK) and had large rectangular holes (150 x 90 mm) cut in the base and lid. The holes were covered with white muslin, creating taught surfaces that allowed air flow through them. Two 6 cm diameter circles were drawn on the muslin base of each arena in pencil, marking out the area around two experimental positions in each arena.

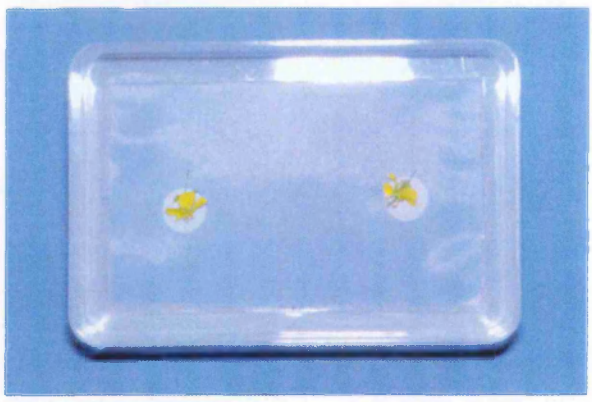


Figure 3.1 Plan view of ventilated arena

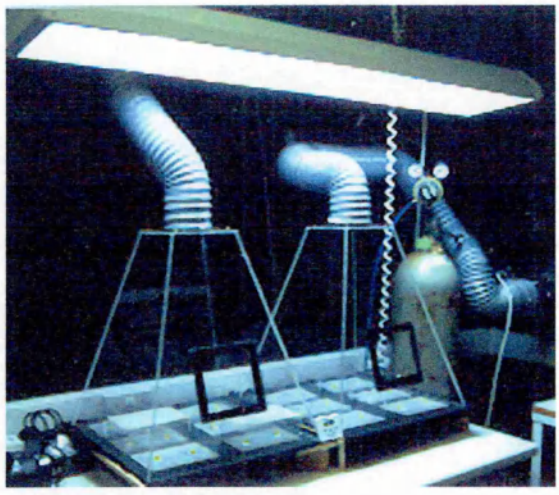


Figure 3.2 Air funnels (each containing 6 arenas)

Six ventilated box arenas were placed inside an air funnel (Figs. 3.2 & 3.3). The air funnel produces steady, vertical air movement (the air moves vertically at 7.6 cm per second through the base of the funnel), effectively separating point sources of odours and preventing odour saturation of the arenas. (The airflow was visualised using smoke plumes from joss-sticks placed inside the arenas and the smoke was seen to move directly upwards through the ventilated arenas with no eddying).

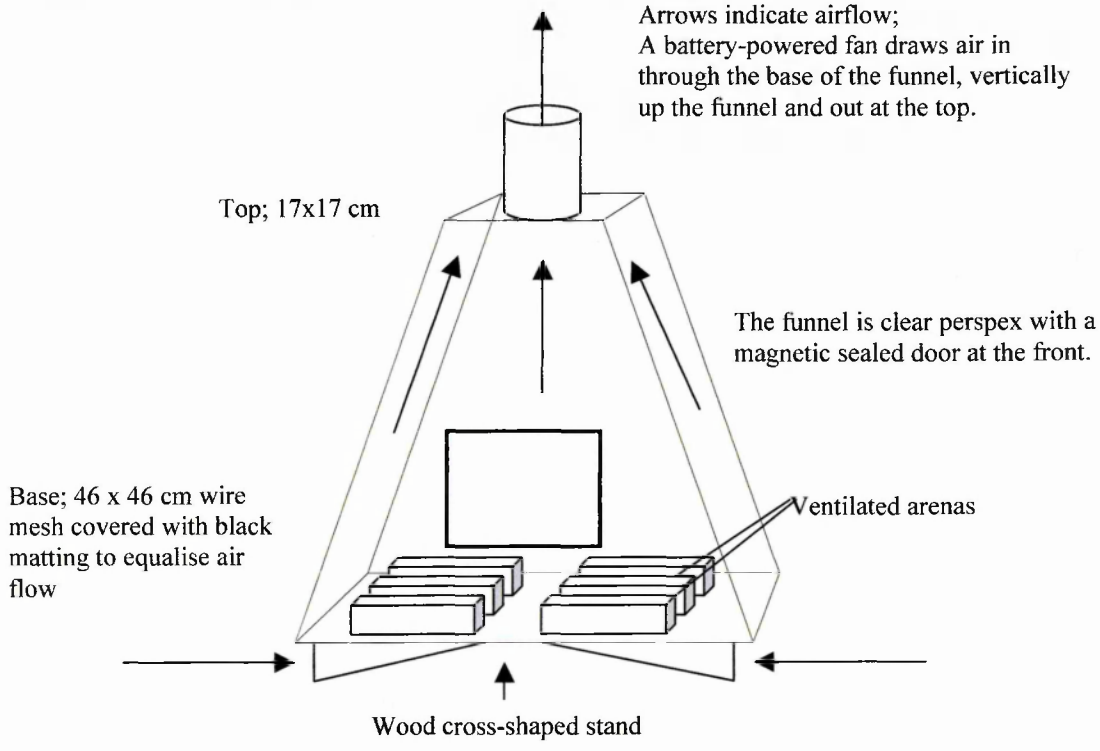


Figure 3.3 Schematic diagram of an airfunnel

3.3.2 Insects

Adult pollen beetles were collected from flowering, spring oilseed rape fields and cultured using the method described in section 2.2. As all the individuals were field collected, it was difficult to establish their physiological state. Each individual could be searching for host plant flowers for food or oviposition sites etc.

Prior to being used in experiments, the beetles were put into clean boxes (soaked in Decon 75 and rinsed in cold water) at 18 °C and starved for 24 hours. On the morning of the bioassays, the insects were acclimatised to the experimental light and temperature conditions for one hour.

3.3.3 Chemical preparation

Eucalyptus, Geranium, Lavender, Peppermint and Ti-tree essential oils (Boots, UK) (see section 2.4) were tested. A series of dilutions (100 %, 10 % and 1 % (v/v)) of each of the essential oils in acetone was produced and stored at 5 °C until needed. At the 100 % concentration, two controls were used; water and rapeseed oil (J Sainsbury plc). However, at the 10 % and 1 % concentrations, acetone was used to dilute the oils and so was used as the only control. Therefore, an extra oil (Ti-tree) was included in the 10% and 1% experiments because there was only one control leaving an extra space inside the air funnel.

3.3.4 Choice-test procedure

The experimental light and temperature conditions were; 19 °C, relative humidity ~ 60 %, with one overhead, 132 Watt, high frequency, polarising white light source (Clearvision Lighting Ltd.). Six ventilated arenas were placed in each air funnel and the equipment was set out within each arena as shown in Figure 3.4. The block of six boxes in each air funnel meant that each of the oils were tested simultaneously, making each set of six boxes one replicate.

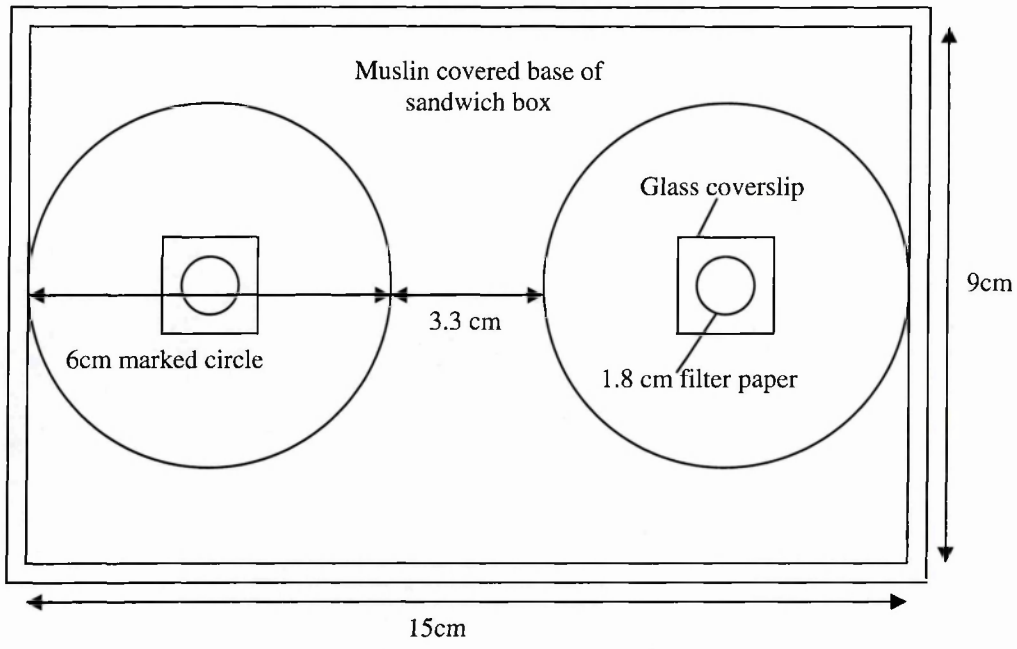


Figure 3.4 Plan view of ventilated box arena showing layout of equipment

A 22 mm x 22 mm glass coverslip was placed on the base of each arena in the centre of each circle. A piece of 1.8 cm Whatman No. 1 filter paper was positioned on each coverslip. Within each arena, 10 µl of essential oil solution was added to one filter paper, described as '**treated**', and 10 µl of water (for the 100 % choices) or acetone (all other choices) was added to the other filter paper, described as '**untreated**' using 10 µl disposable microcaps. Controls were included which presented the choice of **untreated** vs. **untreated**. The position within the funnel and left/right orientation of treatments was rotated for each replicate. After the acetone had evaporated, a single, newly opened oilseed rape flower was placed on each filter paper. The upward airflow through the arenas separated the volatile emissions from the two filter papers in each arena. Ten beetles were introduced into the centre of each arena and the lid was replaced to seal the arena. Males and females were tested separately. After 30 minutes, the number of beetles on each of the flowers was recorded. This was repeated 6 times for each essential oil, and between replicates the arenas were soaked for 24 hours in Decon to prevent any odour contamination.

3.3.5 No-choice procedure

No-choice tests were conducted using the same protocol as detailed above, but only one oilseed rape flower was presented on a piece of filter paper on a glass cover slip in the centre of each arena. These tests were conducted using 10% oils only.

3.3.6 Choice test analysis

Due to low numbers of beetles settling during the experiments, the data for all the replicates were summed (n=1) and statistical analysis performed using these totals. The data were summarised as the total number of beetles in three categories; on the oil-treated flower, on the untreated flower or not on the flowers. Two sets of chi-square statistics were calculated and used to analyse the data for treatment effects.

3.3.6.1 Analysis 1

Chi-square statistics were calculated, using the Pearson formula, to test associations between the treatments (rows) and the choices made by the beetles (columns). These statistics test whether there is an even distribution of choices amongst all treatments. Contingency tables were produced, and chi-square analysis performed using Genstat.

3.3.6.2 Analysis 2

The data were analysed a second time to investigate whether there were any differences amongst the non-host odours on their effects on the beetle's choices. The control (acetone/water/rapeseed) data were excluded to allow comparisons amongst the treated choice tests. New contingency tables were produced and χ^2 statistics were calculated.

3.3.6.3 Repellency Values

Repellency values were calculated according to the formula in Scheffler (1993). The value was calculated using the data for the number of beetles on the treated flower (T) and the number of beetles on the untreated flower (U). The repellency value (RV) = U/(U+T). RVs were calculated for each non-host odour at each of the concentrations.

3.3.7 No-choice test analysis

The no-choice test data were analysed using the same two sets of chi-square tests (3.3.6.1 and 3.3.6.2), but the two choices in the contingency table were limited to **treated** or **not on the flower.**

3.4 RESULTS

3.4.1 Choice test results

Overall, low numbers of beetles (~35-40 %) were on the flowers after 30 minutes. Figures 3.5a, b & c and Figures 3.6a, b & c show the total number of male and female beetles respectively from all the 6 replicates alighting on the treated and untreated flowers.

3.4.1.1 Choice test results – analysis 1

To illustrate how the chi-square was done, Table 3.1 shows the data from the 100 % oil choice tests using male pollen beetles. The data are shown for all the oils (treatments) as the total number of male beetles on the treated flower, untreated flower or were not on the flowers. The total number of beetles per treatment is 60 (the sum of 10 beetles in 6 replicates). The degrees of freedom are calculated as follows; (No. rows -1) x (No. columns-1) = 10.

Table 3.1 Contingency table for analysis 1; number of male beetles on each treatment at 100 % concentration. df = 10, χ^2=86.78, p<0.001. [c] denotes the controls.

100 %	Number of male beetles on:			
Treatment oil:	**Treated**	**Untreated**	**Not on flowers**	**Totals**
Rapeseed [c]	17	17	26	60
Water [c]	19	21	20	60
Eucalyptus	1	38	21	60
Geranium	0	26	34	60
Lavender	0	35	25	60
Peppermint	1	22	37	60
Totals	38	159	163	360

The p values from the chi-square tests, used to analyse the data for the males and females at 100 %, 10 % and 1 % concentrations, are shown on Figures 3.5a, b & c and Figures 3.6a, b & c.

The first analysis compared the distribution of choices made by the beetles (treated flower, untreated flower or neither) for all odours, including a control. In all tests, there was a

significant association between the beetles' choices and the treatment (see p-values on Figures 3.5a, b & c and Figures 3.6a, b & c). This is mainly due to the inclusion of the rapeseed, water and acetone control treatments. The data clearly show, that there was a difference in the choices made by the insects in the presence of non-host plant odours - i.e. when presented with a choice, the beetles significantly preferred the untreated flower to the flower treated with non-host odour.

3.4.1.2 Choice test results – analysis 2

The second analysis compared the distribution of choices made by the beetles (treated flower, untreated flower or not on the flowers) for all oils, without a control. This investigated whether the non-host odours have different effects on the beetles' responses.

As an example of how the chi-square was calculated, Table 3.2 shows the number of male beetles on the treated flower, untreated flower as well as those not on the flowers. These data are the same as those in Table 3.1, however, the control choice tests (rapeseed and water) have been excluded. The total number of beetles per treatment is 60 (the sum of 10 beetles in 6 replicates). The degrees of freedom are calculated as follows; (No. rows -1) x (No. columns-1) = 6.

Table 3.2 Contingency table for analysis 2; number of male beetles on each treatment at 100 % concentration, df = 6, χ^2=13.35, p=0.038.

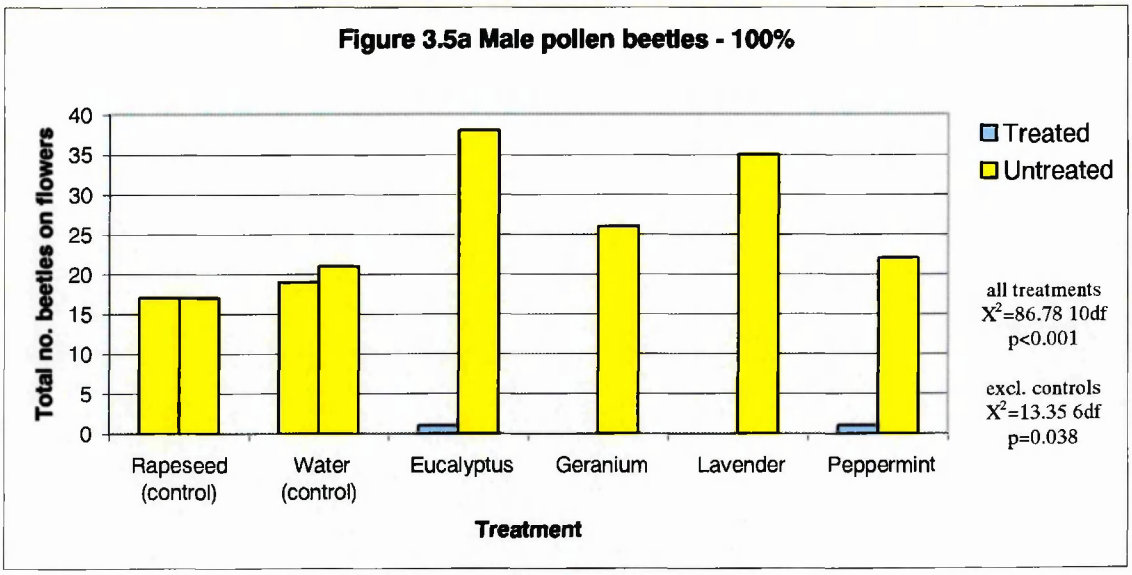
100 %	Number of male beetles on:			
Treatment oil:	**Treated**	**Untreated**	**Not on flowers**	**Totals**
Eucalyptus	1	38	21	60
Geranium	0	26	34	60
Lavender	0	35	25	60
Peppermint	1	22	37	60
Totals	2	121	117	240

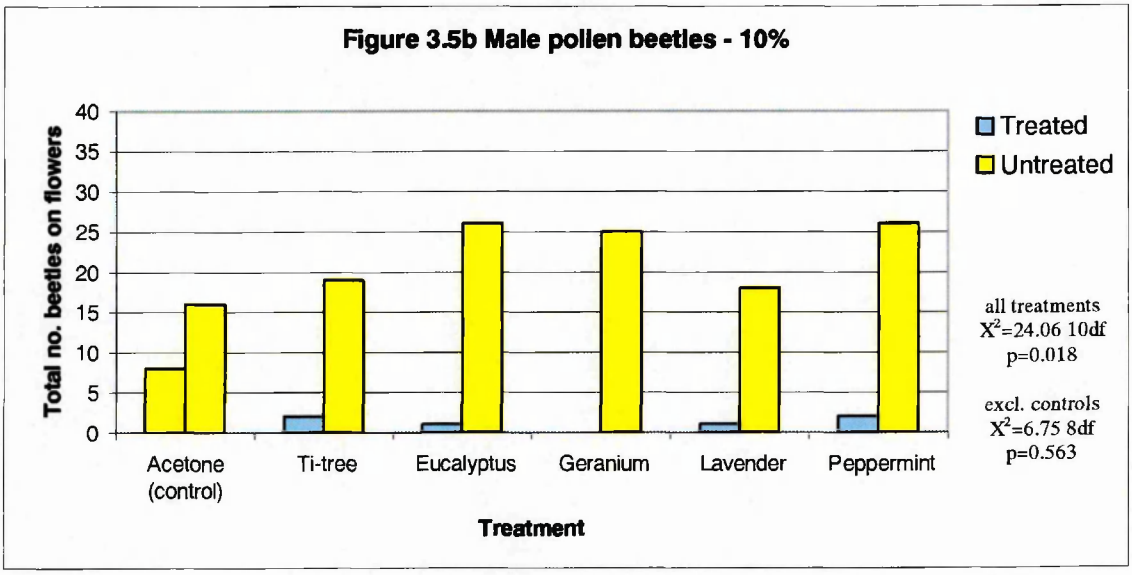
The p values from the chi-square tests, used to analyse the data for the males and females at 100 %, 10 % and 1 % concentrations, are shown on Figures 3.5a, b & c and Figures 3.6a, b & c.

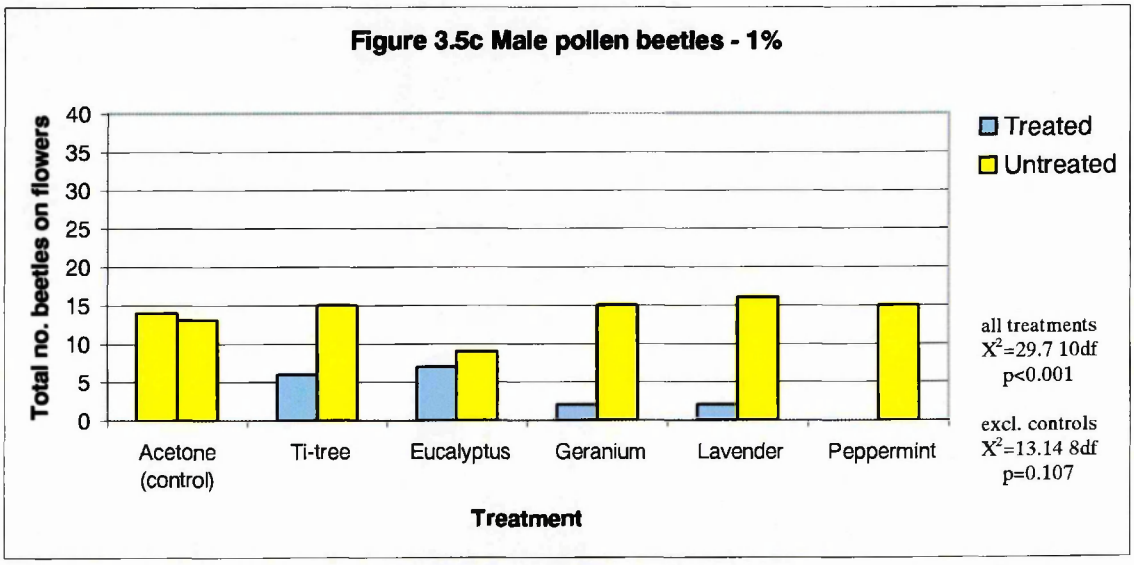
At the highest concentration (100 %) the males showed variability in their responses to the different treatments (p=0.038), however at the lower concentrations of 10% (p=0.563) and 1 % (p=0.107) there were no significant differences between the treatments. The numbers of beetles on the flowers were low in the 10 % and 1 % experiments, which may partly explain why no significant differences were detected.

At 100 % concentration the oils all had similar effects on the female beetles' responses (p=0.806), i.e. all the odours were equally avoided. Reduction in the concentration to 10 % produced significant differences in the choices made by the beetles in response to the different non-host odours (p=0.033). But at 1 % concentration there was no significant difference (p=0.062) as most of the treatments had few beetles on each flower, with the exception of lavender.

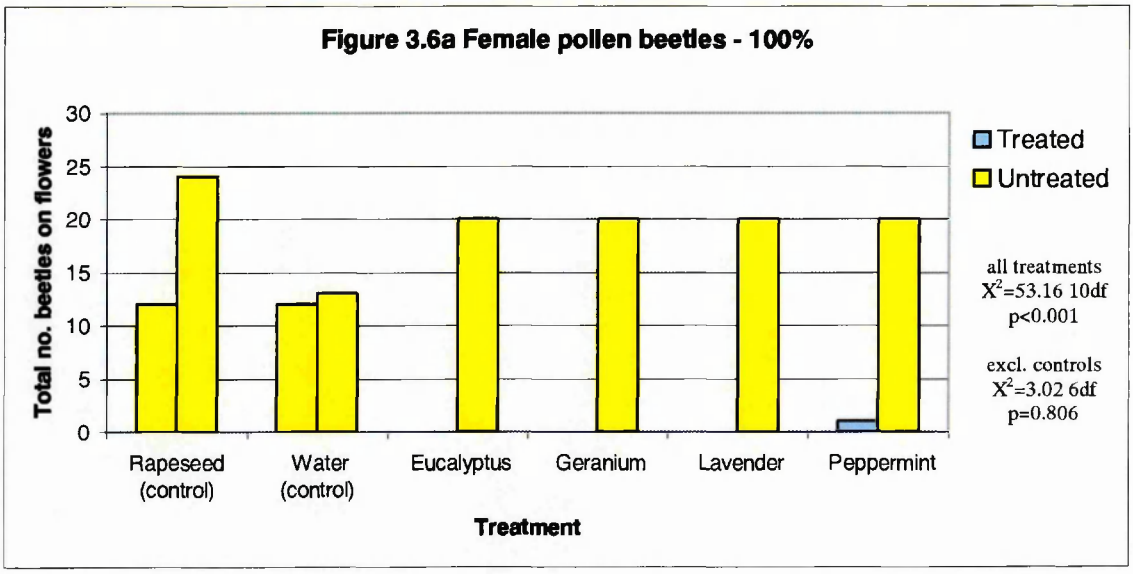
However, similar overall trends were seen in the males' responses as the females; as the concentration decreases, the number of beetles on the treated flower increased.

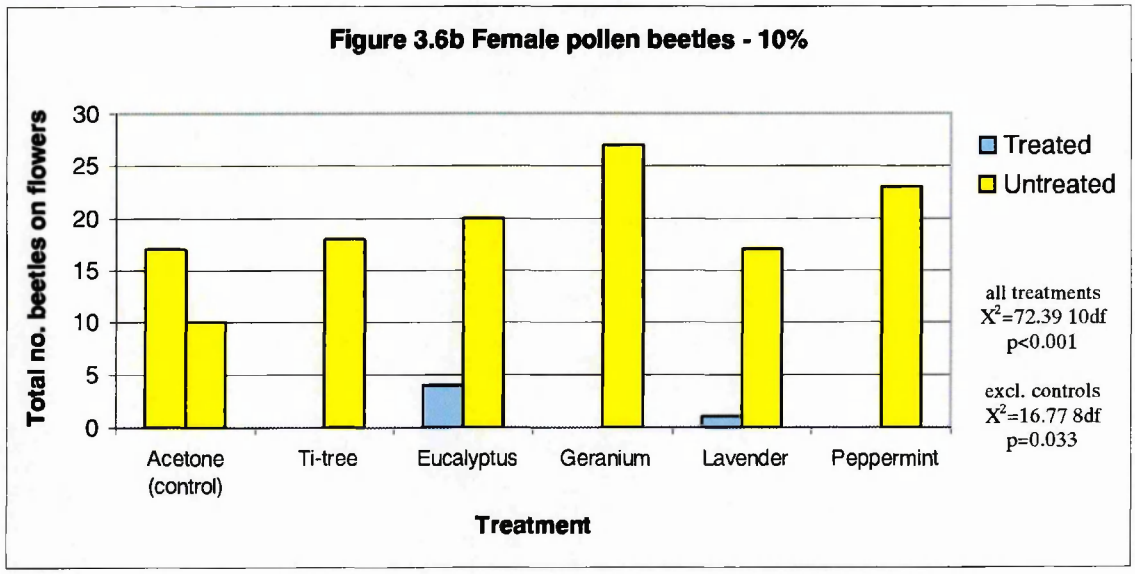


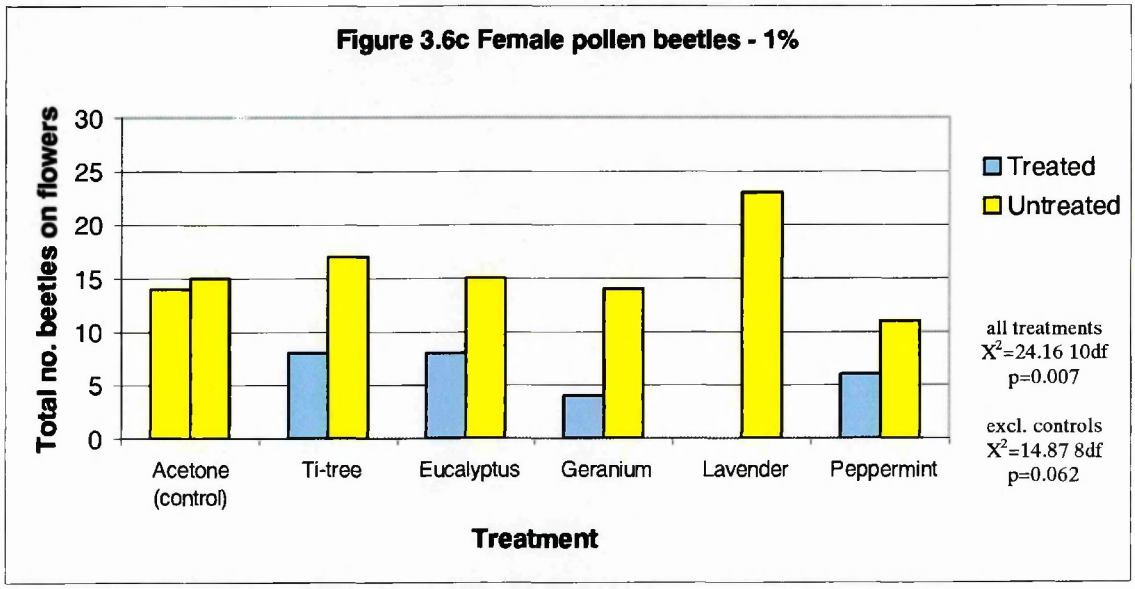




Figures 3.5 a, b & c. Numbers of male beetles on the treated and untreated flowers at 100%, 10% & 1% concentrations respectively







Figures 3.6 a, b & c. Numbers of female beetles on the treated and untreated flowers at 100%, 10% & 1% concentrations respectively

3.4.1.3 Repellency Values

High Repellency Values indicate repellency. RVs were calculated for each odour at each concentration (Figures 3.7 and 3.8). In general, the RV increased with increasing concentration for all the odours. Differences amongst the odours were evident at the lower concentrations for both males and females.

It is useful to compare the non-host odours using a single value i.e. the mean RV for each odour across all concentrations (Table 3.3). From these values, it is clear that Eucalyptus (mean RV=0.83) and Ti-tree (mean RV=0.82) are the least repellent, whereas lavender (mean RV=0.96) is the most repellent overall.

Table 3.3 Mean repellency values for each non-host plant odour

RVs	Female Mean	Male Mean	Overall mean
Eucalyptus	0.83	0.83	0.83
Geranium	0.93	0.96	0.94
Lavender	0.98	0.95	0.96
Peppermint	0.87	0.96	0.91
Ti-tree	0.84	0.81	0.82

Figure 3.7 Female pollen beetles Repellency Values
No. of beetles on the untreated flower/total no. beetles on flowers

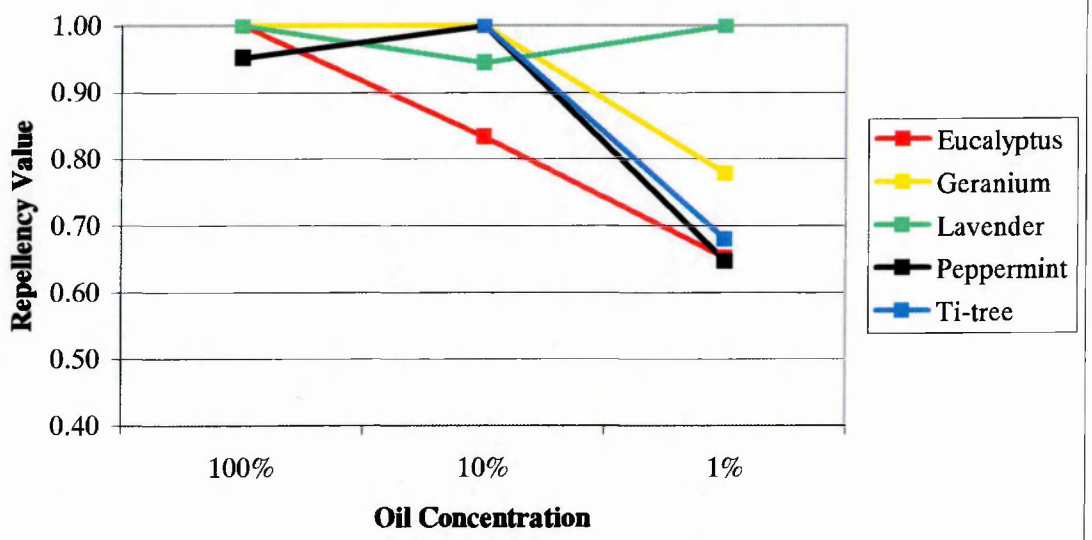
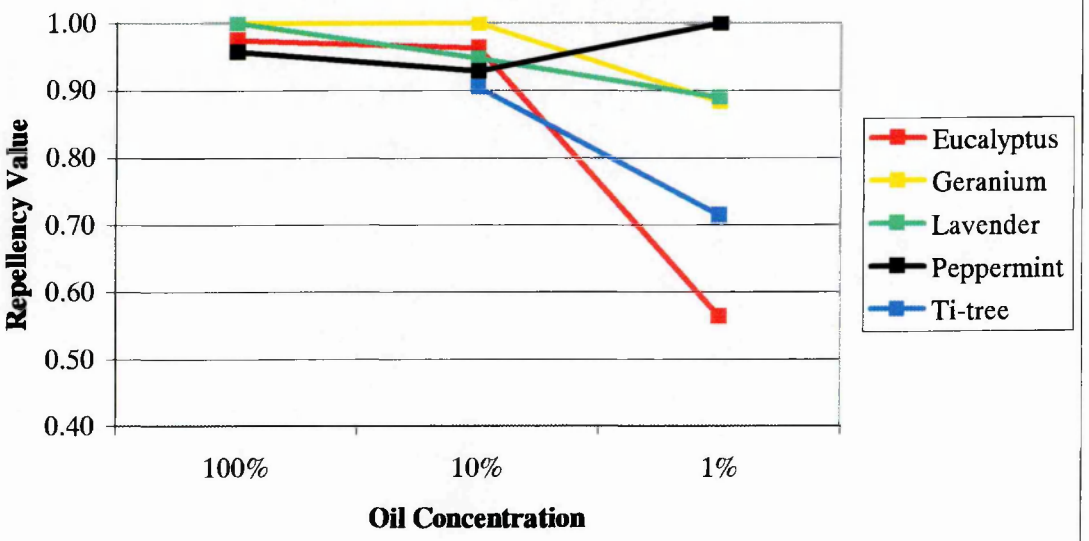


Figure 3.8 Male pollen beetles Repellency Values
No. of beetles on the untreated flower/total no. beetles on flowers



3.4.2 No-choice test results

The results from the no-choice tests (Figures 3.9 & 3.10) show that the control (acetone) treatment had a significantly different effect on the beetles' responses compared to the addition of non-host plant odours for both the males and females ($p<0.001$). In the second analysis, the non-host odours again varied in their effects on the beetles (females $p=0.033$, males $p=0.044$) with a similar order of effectiveness to that seen in the choice tests; eucalyptus was least avoided (most colonised), followed by peppermint, geranium, ti-tree, then lavender.

In both the choice and no-choice tests, the lavender treated flowers appeared to have fewer beetles.

Figure 3.9 Number of females (n=60) on the flower in the no-choice bioassays

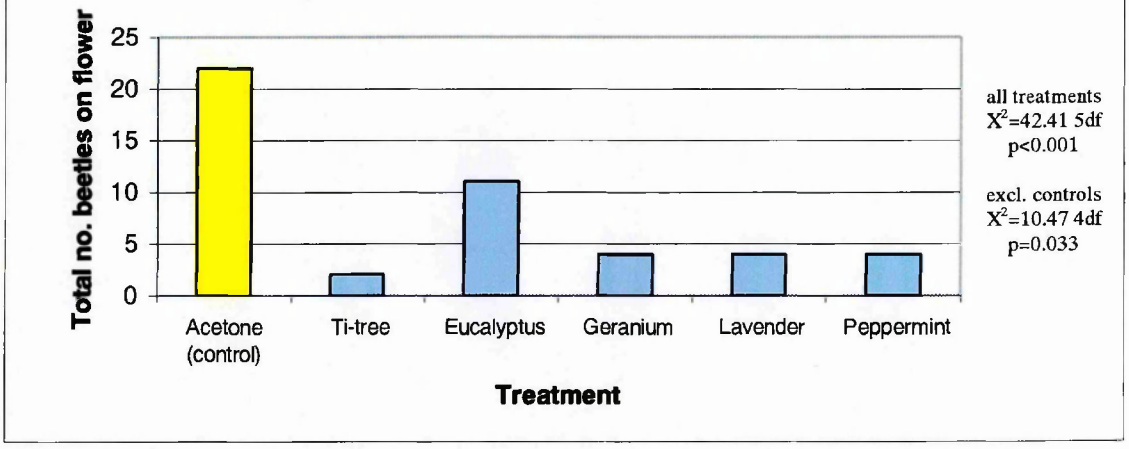
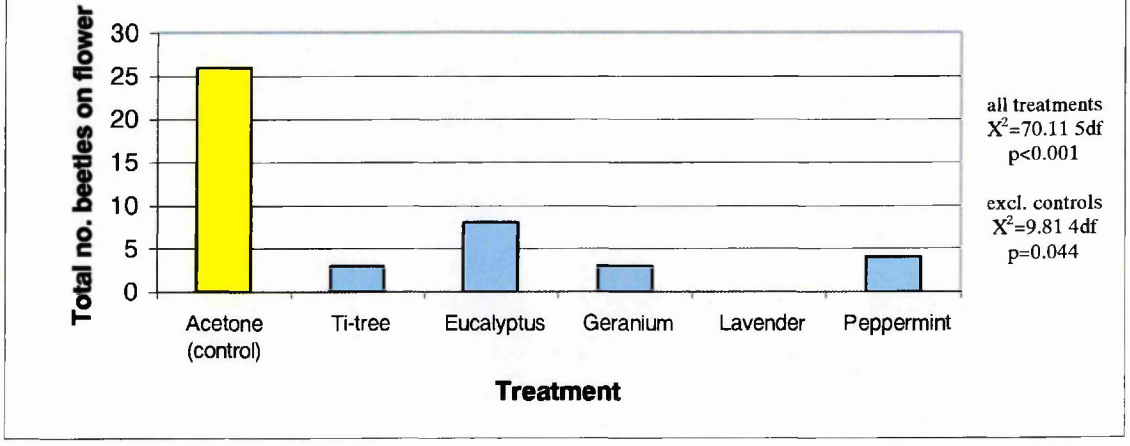


Figure 3.10 Number of males (n=60) on the flower in the no-choice bioassays



3.5 DISCUSSION

The results from these arena bioassays show that non-host plant essential oils are effective at reducing counts of *M. aeneus* on isolated host flowers. When presented with a choice of two oilseed rape flowers, treated and untreated, the significant majority of males and females were found on the untreated flower. These results show that this novel method, using a vertical airflow through enclosed arenas, is an effective method of separating two odour fields and providing two distinct odour choices.

The method provided visually similar host flowers, yet the insects were able to distinguish between them. Therefore, it can be concluded that the insects were likely to be using olfactory cues in decision-making during host colonisation. These results support findings from both laboratory (Evans & Allen-Williams, 1994) and field (Blight & Smart, 1999; Smart & Blight, 2000) studies where both host-related visual *and* olfactory cues were shown to influence the behaviour of *M. aeneus*.

These experiments form the preliminary stage in the process of semiochemical research described by Poppy (1991) where observations of behaviour are required before moving on to investigate the chemistry (Chapter 5) and precise mechanisms of the behaviour (Chapter 6), and ultimately should be followed by applied research using field trials (Chapter 7).

Overall, the flowers treated with non-host plant essential oils were avoided at all concentrations tested, compared to the untreated flowers. The avoidance behaviour seen in these bioassays indicates that the essential oils are acting as masking agents or repellents to the beetles (Section 1.2). Overall, both sexes responded in a similar manner, however in Chapter 4, some sexual differences in their response to non-host odour were detected. There were often significant differences in the distribution of beetles between differently treated flowers in the choice tests, indicating that the oils were not equally avoided. Eucalyptus was avoided, but this effect was the weakest, whereas lavender proved to be most consistently avoided in both the choice and no-choice tests. This demonstrates that the concentrations used in these tests were appropriate to identify differences in responses by the beetles to these oils.

An effect of concentration of the essential oils was evident - the higher concentrations were the most repellent. In order to understand the biological relevance of these findings, it is important to note that some compounds can cause attraction at one concentration and repellence at another (Dethier, 1947). Therefore, to avoid false positives, the most strongly avoided treatment at the lowest concentration indicates the most effective repellent. Lavender essential oil was the most avoided at the lowest concentration and was therefore selected for further behavioural testing in Chapter 4.

The no-choice test proved that this avoidance behaviour in *M. aeneus* was not over-ridden during the 30-minute observation by the need to locate a host. Again, eucalyptus oil repellency was the most readily overcome whereas lavender oil remained the most repellent. A longer sampling time would have provided information about the duration of the repellent effect, but would need careful monitoring to be able to distinguish between habituation of the beetles to the odour and a possible change in the odour profile over time. These bioassays were designed to screen for potential repellents, and therefore the 30 minute observation time was sufficient to test each individual beetle's initial response to odours - if they had a neutral effect, the beetles would not have been seen to make such a clear choice.

These bioassays have tested the initial behavioural response of *M. aeneus* to very close-range exposure to a mixture of volatiles. There is strong evidence that some of the volatiles in the mixture have the effect of causing *M. aeneus* to avoid the treated flower. It is unclear from these assays how the non-host volatiles are causing this effect. There are two possible ways in which they could be acting on the beetles. Firstly, the non-host plant odours could have masked the attractive host plant odours, preventing the beetles from recognising the treated flower as a host. This has been suggested as the mode of action of non-host plant volatiles on the behaviour of aphids (Nottingham *et al.*, 1991) and Colorado potato beetle (Thiery & Visser, 1986). Alternatively, or in conjunction, the non-host odours could have a direct repellent effect on the insects (Glinwood & Pettersson, 2000a) i.e. the non-host odour alone is avoided by the insects. Whatever the mechanism, it is clear that non-host plants do contain volatile compounds that elicit avoidance responses in *M. aeneus*.

Lavender, *Lavandula angustifolia* (Lamiaceae), is not a host plant of *M. aeneus*, and in tests the beetles actively avoided landing on lavender plants (personal observation).

Therefore, the use of lavender essential oil is a way of providing a consistent 'non-host' signal to investigate the specific behaviours behind the insects' responses to non-host plant volatiles.

Lavender essential oil needs to be tested to determine whether it is truly repellent to *M. aeneus* or whether it simply masked the attractive odours from the oilseed rape flowers in these bioassays. These tests are the subject of Chapter 4.

CHAPTER 4. CHARACTERISATION OF THE RESPONSE OF *MELIGETHES AENEUS* TO NON-HOST PLANT ODOURS

4.1 INTRODUCTION

Non-host plants are avoided by phytophagous insects and can affect their host location and acceptance behaviour (Section 1.4.9). Visually, non-host plants may reduce the contrast between the host and its background, or simply hide the plants from view (Bernays & Chapman, 1994). By olfactory detection, the odour from non-host plants can disrupt the attraction of an insect to its host plant by simply masking the host odours, eliminating positive attraction to the host (Thiery & Visser, 1986). But non-host plant odours can also have a further effect by being repellent, in the sense of actually causing avoidance by the insect.

Both of these olfactory mechanisms have been implicated in aphid responses to mixtures of host and non-host odours. Nottingham (1991) showed that plant volatiles may play a role in host location by aphids, and that host-plant attraction can be masked by the presence of non-host volatiles. Olfactory cells on the aphids antennae responded electrophysiologically to compounds from non-host plants which, depending on concentration, led to repellency or masking of host attraction. Additionally, post-alighting inspections of the plant enable the detection of inappropriate physiology, nutritional indicators and toxic secondary chemicals during colonisation or feeding. This has been demonstrated in the aphid *Aphis fabae* Scop. which uses methyl salicylate and (-)-(1R,5S)-myrtenal as indicators of nutritionally unsuitable or non-host plants (Hardie *et al.*, 1994). Also, in diverse plant assemblages host and non-host leaves can overlap, thereby increasing the chance that the insect will land on a non-host leaf, disrupting the process of host acceptance (Finch & Collier, 2000).

Deterrents are non-volatile chemicals that insects experience on landing and attempting to feed or oviposit on the plant. These are the final factor involved in non-host recognition and include feeding deterrents such as iridoid glucosides (Puttick & Bowers, 1988).

The experiments in chapter 3 demonstrated that pollen beetles avoid oilseed rape flowers treated with non-host plant essential oils. In summary, treatment of an oilseed rape flower

with lavender oil was most effective at reducing the number of insects on that flower. It is believed that this response is most likely to be due to volatile olfactory cues from the oil, but an experiment using an olfactometer was performed in this chapter to confirm whether the insects' response to lavender oil was consistent when visual or contact cues were eliminated (Experiments 1 and 2).

The results of the experiments in chapter 3 do not indicate whether the lavender oil is just masking (or overriding) the attractiveness of the floral volatiles from the oilseed rape flower, or whether the lavender oil is having an additionally repellent effect on these insects. In order to establish whether lavender oil alone causes a repellent response, experiments were conducted in this chapter to examine the responses of *M. aeneus* to lavender oil without the visual or olfactory stimuli from oilseed rape flowers (Experiment 3).

If the non-host odour is actually having a repellent effect, in addition to masking the host volatiles, this may increase the efficiency of the odour in pest control. The benefit of using a repellent is that, in theory, the odour can prevent colonisation of the crop completely. If the repellent is highly active, it can cause the flying insect within the crop to change behaviour and induce movement away from the crop. Computer simulation models of insect population patterns show repellent odours reduce pest numbers from the crop most effectively if the odour induces flight away from the field (R. Potting personal communication). This has the effect of removing the pests without concentrating them into other areas of the main crop.

Olfactometers have been used in many previous studies investigating the response of a whole range of insects to olfactory cues. Designs include linear track (Sakuma & Fukami, 1985) and Y-tube (e.g. Ruther & Thiemann, 1997) olfactometers, which both present the insect with two choices, and the 4-arm olfactometer (Pettersson, 1970) which can present four choices at once. The 4-arm olfactometer (see section 4.3.2) is a modification of a design by Pettersson (1970) and was chosen for use in this study. The advantage of the 4-arm olfactometer is that, when measuring repellency, a reduction in the number of individuals required for testing can be achieved by offering the test odour through three arms simultaneously and leaving one arm blank. This is because if the test insect is not responding to the test odour, you would expect 25% to end up in the blank field as opposed

to 50% doing so in an olfactometer with two arms. Thus, fewer insects are needed to obtain the same statistical power (Vet *et al.*, 1983).

Besides lavender, two other compounds were also tested for their effect on *M. aeneus* using the 4-arm olfactometer. Appendix 1 details a botanical survey conducted to identify non-host plants of *M. aeneus*. From this, pineapple mayweed, *Chamomilla suaveolens* was chosen and water-distilled (Williams, 1996) extract the essential oil. The justification for this selection was that a related species, scentless mayweed, *Matricaria perforata*, was heavily colonised by pollen beetles whereas none were seen on pineapple mayweed throughout the season. Additionally, pineapple mayweed releases volatiles that could act as a repellent, although visual cues were not assessed in this survey. An extract of gum haggar, *Commiphora erythraea* was also tested. Gum haggar extract has known repellent properties against aphids (J.A. Pickett, personal communication) and ticks (Carroll *et al.*, 1989). The gum haggar extract was produced and purified by M. Birkett at Rothamsted.

4.2 AIMS

1. To investigate whether the response to host plant odour is altered by the presence of lavender oil, after visual and contact cues have been eliminated (Experiments 1 & 2).
2. To determine whether lavender, pineapple mayweed and gum haggar essential oils elicit a response in *M. aeneus* when presented on their own (Experiments 3, 4 & 5).
3. To investigate whether these effects are similar for both sexes of *M. aeneus*.

4.3 MATERIALS AND METHODS

4.3.1 The 4-arm olfactometer

The 4-arm olfactometer is designed for investigating olfactory responses of small insects placed in a chamber in which they can walk around freely (Pettersson, 1970). The equipment produces four distinct odour fields, one in each quadrant of the chamber. A pump sucks air out through the central hole, drawing air equally through the four arms. The rate of airflow is regulated using a flowmeter. There is mixing of the odours in the central part of the olfactometer, but there are clear boundaries at the entrances to the arms.

4.3.2 Equipment

The 4-arm olfactometer chamber is made from three pieces of transparent perspex held together with plastic nuts and bolts. The central piece is circular with walled edges into which fit the top and bottom pieces, and it has a four-pointed star-shaped space cut through the centre (Figure 4.1). At the end of each point there is a hole through the wall, into which the arms are fitted. The top and bottom pieces are flat, circular perspex with holes for the fastening bolts, the bottom piece also has a small hole in the centre. The hole in the bottom of the olfactometer is the air extraction point and the beetle entry point.

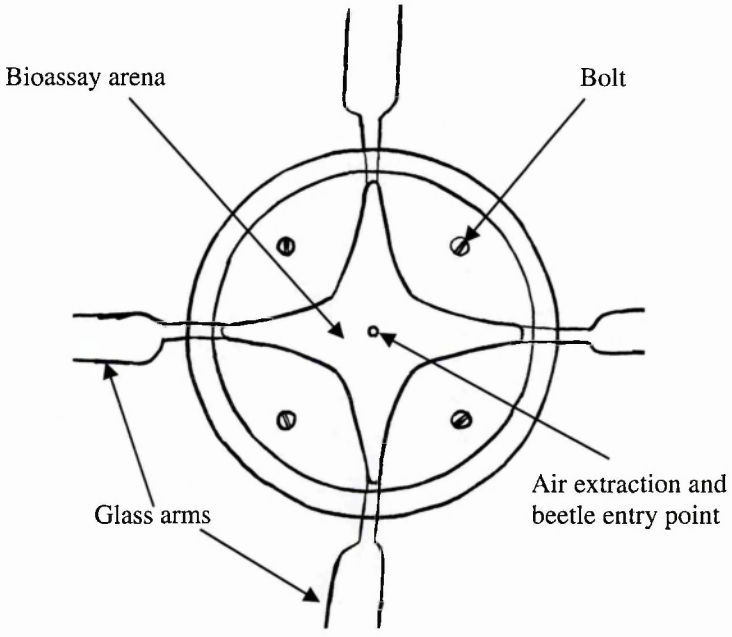


Figure 4.1. Plan view of the 4-arm olfactometer

The arms are pieces of glass tubing narrowed at one end. The narrow ends are fitted into small holes in the side of the olfactometer - this connection is made air-tight using Teflon tape. A piece of muslin covers the narrow end of the glass arm to prevent the beetle entering it.

For this experiment, the olfactometer was placed on a paper-covered, raised platform with the central entry hole facing down. The pump tubing was connected to the olfactometer from below to avoid the pump tubing shading the arena. The connection was made using a cut-off pipette tip pushed into the end of the tubing leading to the pump; see Figure 4.2.

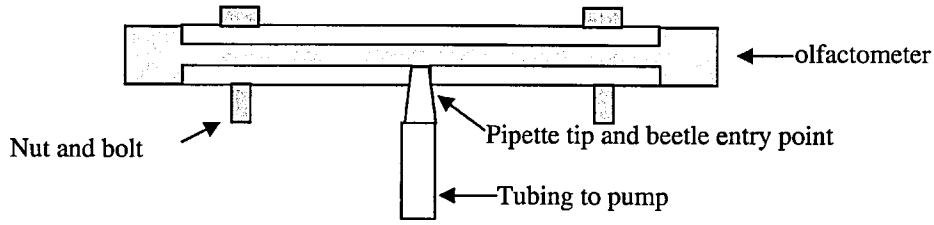


Figure 4.2 Section through middle of olfactometer showing combined insect entry hole and exhaustion tube

4.3.3 Procedure

The experimental light and temperature conditions were; 19 °C, Relative humidity ~ 60-70% with one overhead, 132 Watt, high frequency, polarising white light source (Clearvision Lighting Ltd.). The airflow rate in the olfactometer was fixed at 400 ml/min using the flow meter. On the morning of the bioassays, the insects were placed into a dark box to ensure a strong phototactic response at the start of the experiment.

Filter papers were used as a release source of either essential oil or acetone (control). 1.8 cm diameter filter papers were placed in the wide end of each glass arm of the olfactometer. Ten µl of essential oil or acetone were pipetted onto each filter paper using a microcap (see individual experiments; sections 4.3.5 for treatments).

An individual beetle was taken from the dark box and placed in the pipette tip. The distal end of the pipette tip was covered with muslin and inserted into the tubing. The near end of the pipette tip was pushed into the entry hole on the underside of the olfactometer. The beetle was left to walk up the tip and into the olfactometer following a light gradient. The individual was rejected if it was inactive and did not enter the olfactometer within five minutes. As the beetle entered the olfactometer, the pump was switched on and the behavioural recording began.

The beetle was observed for five minutes and its position within the olfactometer was continuously recorded using the Observer software (Version 3.0, Noldus Information Technology, Wageningen, 1996). Each replicate was conducted using a clean olfactometer (washed in hot water with Teepol, rinsed in 70% ethanol and then rinsed in cold, distilled water) to prevent orientation to any cues left by the essential oil volatiles or the previous

beetle. Also, the position of the control arm was changed each time to prevent orientation to external cues, although these were minimised as far as possible by conducting the experiments in a room painted throughout with matt black paint.

4.3.4 Insects

Adult pollen beetles, *M. aeneus,* were collected from flowering spring oilseed rape field crops in late July/August 2000 (experiment 3) and winter oilseed rape in April/May 2001 (all the other experiments). The beetles were kept in culture (see section 2.2). Prior to being used in experiments, the insects were put into clean sandwich boxes at 18 °C and starved for 24 hours.

4.3.5 Experiments

Beetles' attraction to the odour of oilseed rape flowers was confirmed and then challenged using lavender oil. Beetles' responses to lavender, gum haggar and pineapple mayweed oil were also tested. The lavender experiment was repeated three times, firstly to compare the responses to two different supplies of lavender oil and secondly to test whether the same response occurs when tested at different times during the beetles' lifecycle.

4.3.5.1 Experiment 1. Responses to oilseed rape flowers

Four freshly cut oilseed rape flowers (from glasshouse-grown plants) were placed in one glass arm of the olfactometer. The other three arms were left empty. Fresh flowers were used for every individual tested. No essential oil was applied to any of the filter papers. 10 female and 10 male beetles were tested. Experiment was conducted at the end of April 2001.

4.3.5.2 Experiment 2. Response to oilseed rape flowers in the presence of lavender oil

Four freshly cut oilseed rape flowers were placed in each of the four glass arms of the olfactometer. Filter paper in 3 of the arms was treated with 1% v/v (8.2mg/ml) lavender oil (Botanix), the fourth arm contained acetone treated filter paper. Fifteen female and 15 male beetles were tested. Experiment conducted at beginning of May 2001.

4.3.5.3 Experiment 3a. Responses to lavender oil (Boots)

Lavender essential oil (Boots, see section 2.4) was diluted in acetone to produce a 10% v/v solution. Lavender oil of the same concentration was applied to filter papers in 3 of the

olfactometer arms, and the control filter paper in the fourth arm was treated with acetone. 15 female and 15 male beetles were tested. This was repeated for concentrations of 1% and 0.1% v/v (8.2 mg/ml). This experiment was conducted in August 2000.

Checks were made to ensure that there was no orientation to external cues by running a blank test (all 4 arms with acetone controls) for both sexes each day.

4.3.5.4 Experiment 3b. Responses to lavender oil (Botanix)

Lavender essential oil (Botanix, see section 2.4) was diluted in acetone to produce a 1% v/v solution (which equates to 8.2mg/ml). The 1% concentration was chosen for further experiments because it caused a clear change in the insects' behaviour. Lavender oil was applied to filter papers in 3 of the olfactometer arms, and the control filter paper in the fourth arm was treated with acetone. Fifteen female and 15 male beetles were tested for repellency. This experiment was conducted at the beginning of April 2001.

4.3.5.5 Experiment 3c. Responses to lavender oil (Botanix)

Repeat of experiment 3b conducted at the end of April 2001.

4.3.5.6 Experiment 4. Responses to Pineapple mayweed oil

Pineapple mayweed essential oil (see appendix 1) was diluted to 10 mg/ml in acetone. The oil was applied to filter papers in 3 of the olfactometer arms, and the control filter paper in the fourth arm was treated with acetone. Fifteen female and 10 male beetles were tested. Experiment was conducted in the middle of May 2001.

4.3.5.7 Experiment 5. Responses to gum haggar extract

Gum haggar extract was prepared and diluted to 1% v/v in hexane. Gum haggar extract was applied to filter papers in 3 of the olfactometer arms, and the control filter paper in the fourth arm was treated with hexane. Fifteen female and 15 male beetles were tested. Experiment was conducted at the end of April 2002.

4.3.6 Analysis

Observer software (Version 3.0, Noldus Information Technology, Wageningen, 1996) was used to produce summary data for each individual beetle, and the parameters used for analysis were; total time spent in each arm and number of visits to each arm. For each

treatment, the null hypotheses of equal time spent in each arm, and equal number of visits to each arm, were tested using Friedman's non-parametric ANOVA (Siegel & Castellan, 1988) as a procedure in Genstat (5th Edition for Windows, Lawes Agricultural Trust, 2000). Friedman's test has been applied to data from a similar study using a 4-arm olfactometer (Couty *et al.*, 1999).

Friedman's test is a non-parametric ANOVA for analysing a randomised complete block design. The treatments (arms) were ranked for each block (beetle) and the sum of the ranks was calculated for each treatment group over all the blocks. The sum of the squared values of these rank sums was calculated and the test statistic (Fr) was calculated using the equation in Siegel (1988), which was adjusted for ranking ties and used for calculating the significance level. The calculation for the probability value was based on a chi-square approximation.

4.4 RESULTS

The results from experiments 1-5 are shown in Figures 4.3 to 4.20 as the mean time (± standard error) spent or the mean number of visits to each arm of the 4-arm olfactometer, for both male and female pollen beetles. The two treatments tested in each experiment are shown in different colours and all four arms are shown - the order along the x-axis is; control arm, left arm, opposite arm and right arm (relative to the control arm). The actual data are presented here, whereas the analysis was conducted on the ranks of the arms.

4.4.1 Experiment 1. Responses to oilseed rape flowers

This experiment was checking for olfactory attraction of oilseed rape flowers to pollen beetles (Figures 4.3 and 4.4). As expected, females and males spent more time in the oilseed rape treated arm ($p=0.003$ and $p=0.002$ respectively) and visited it more frequently ($p=0.001$ and $p=0.003$ respectively) than the blank arms.

4.4.2 Experiment 2. Response to oilseed rape flowers in the presence of lavender oil

Having established the attraction to oilseed rape flowers (experiment 1), experiment 2 investigated whether lavender affects the pollen beetles response to presence of host plant odours (Figures 4.5 and 4.6). The results were similar to those obtained with lavender alone (experiments 3 a & c). Females and males spent most time in the control arm ($p=0.008$ and $p<0.001$), males visited the control arm more frequently than the treated

arms ($p<0.001$), although the females did not visit the control arm more frequently ($p=0.102$).

4.4.3 Experiment 3a. Responses to lavender oil (Boots)

The data from the blank tests were very variable (see Tables 4.1 and 4.2). Male pollen beetles spent equal time in each arm of the olfactometer ($p=0.214$) and visited each arm an equal number of times ($p=0.271$). However, female pollen beetles did not spend equal time in each arm ($p=0.043$); they spent longer in arm 4 but did not visit it more frequently ($p=0.148$).

Table 4.1 Mean time (seconds) in each arm during blank tests

Sex N=7	Arm 1	Arm 2	Arm 3	Arm 4	Friedman statistic	p-value (3DF)
Female	69.43	30.00	57.57	121.29	8.17	0.043
Male	50.57	44.71	69.86	75.43	4.48	0.214

Table 4.2 Mean number of visits to each arm during blank tests

Sex N=7	Arm 1	Arm 2	Arm 3	Arm 4	Friedman statistic	p-value (3DF)
Female	2.00	1.43	1.43	2.43	5.35	0.148
Male	2.00	2.14	2.71	3.29	3.91	0.271

When testing with lavender essential oil, female pollen beetles spent significantly more time in the control arm at all concentrations of lavender essential oil (0.1% $p=0.031$, 1% $p<0.001$, 10% $p=0.002$) (Figures 4.7, 4.9 and 4.11). Male pollen beetles showed a significant preference for the control arm in the 10% lavender tests ($p=0.001$) (Figure 4.11). Males spent the same amount of time in the control arm as the test arms with lavender at 0.1% and 1% (Figures 4.7 and 4.9) ($p=0.295$ and $p=0.234$ respectively).

The number of visits to each arm differs with the sex of the beetle. Male pollen beetles made a similar number of visits to each arm for all concentrations of lavender (Figures 4.8, 4.10 and 4.12). Females made a similar number of visits to all arms for the 0.1% lavender treatment ($p=0.133$), but the control arm was visited more frequently in the 1% ($p<0.001$) and 10% ($p=0.004$) treatments.

The total number of visits to any arm of the olfactometer gives an indication of overall activity while in the olfactometer. As Table 4.3 shows, the males (mean=8.47 total visits) were more active than the females (mean=5.42 total visits) throughout the experiment.

Table 4.3 Mean number of visits to all arms during the 5 minutes in the essential oil tests at all three concentrations (Boots lavender oil)

Sex N=15	Conc.	Mean no. visits to all arms	Mean no. visits over all treatments
Female	0.1%	6.00	
Female	1%	4.2	
Female	10%	6.07	**5.42**
Male	0.1%	8.93	
Male	1%	10	
Male	10%	6.47	**8.47**

4.4.4 Experiment 3b. Responses to lavender oil (Botanix)

This was a repeat of experiment 3a but using a different source of lavender oil and testing at 1% only. The results are very different from experiment 3a (Figures 4.13 and 4.14). Neither the females ($p=0.302$) nor the males ($p=0.697$) spent longer in the control arm compared to the lavender-treated arms. The frequency of visits to each arm were also similar for both treatments; females ($p=0.431$), males ($p=0.205$).

4.4.5 Experiment 3c. Responses to lavender oil (Botanix)

This was a repeat of experiment 3b using 1% Botanix lavender oil but conducted 3 weeks later. This time, the results were very similar to the results obtained in experiment 3a (Figures 4.15 and 4.16). The females spent significantly more time in the control arm ($p<0.001$) and visited it more frequently ($p=0.001$). The males spent more time in the control arm ($p=0.028$) but did not visit it the most frequently ($p=0.073$).

4.4.6 Experiment 4. Responses to pineapple mayweed oil

There was no evidence of avoidance of the *C. suaveolens* treated arms by the pollen beetles (Figures 4.17 and 4.18). The females spent the same amount of time ($p=0.935$) and visited ($p=0.837$) the control arm as frequently as the treated arms. The results were similar for the males ($p=0.581$ and $p=0.420$).

4.4.7 Experiment 5. Responses to gum haggar extract

There was no evidence of avoidance of the gum haggar treated arms by the pollen beetles (Figures 4.19 and 4.20). The females spent the same amount of time ($p=0.115$) and visited ($p=0.132$) the control arm as frequently as the treated arms. The results were similar for the males ($p=0.116$ and $p=0.686$).

Figure 4.3 Mean time spent in each arm (±SE)
Experiment 1. Oilseed rape (1 arm) vs Control (3 arms)

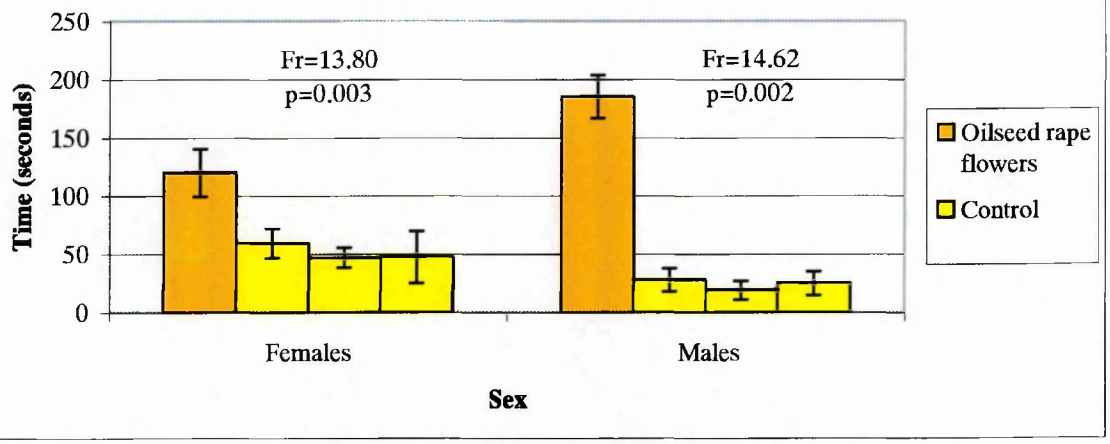


Figure 4.4 Mean number of visits to each arm
Experiment 1. Oilseed rape (1 arm) vs Control (3 arms)

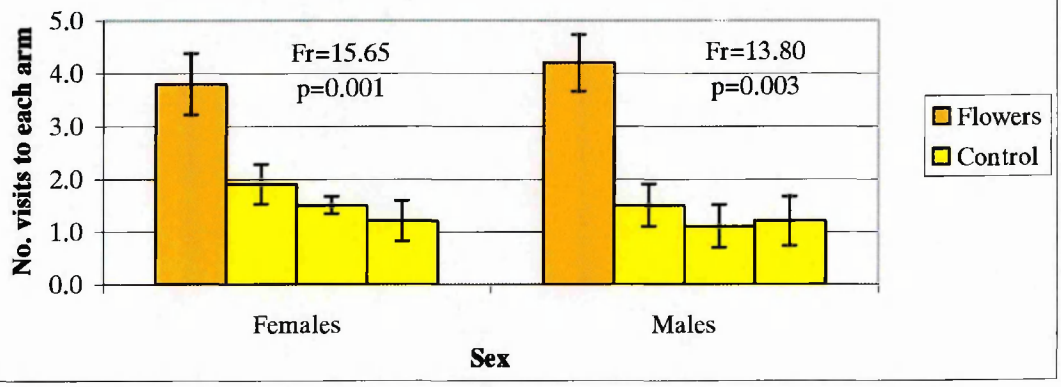


Figure 4.5 Mean time spent in each arm (±SE)
Experiment 2. Oilseed rape (1 arm) vs oilseed rape + 1% lavender (3 arms)

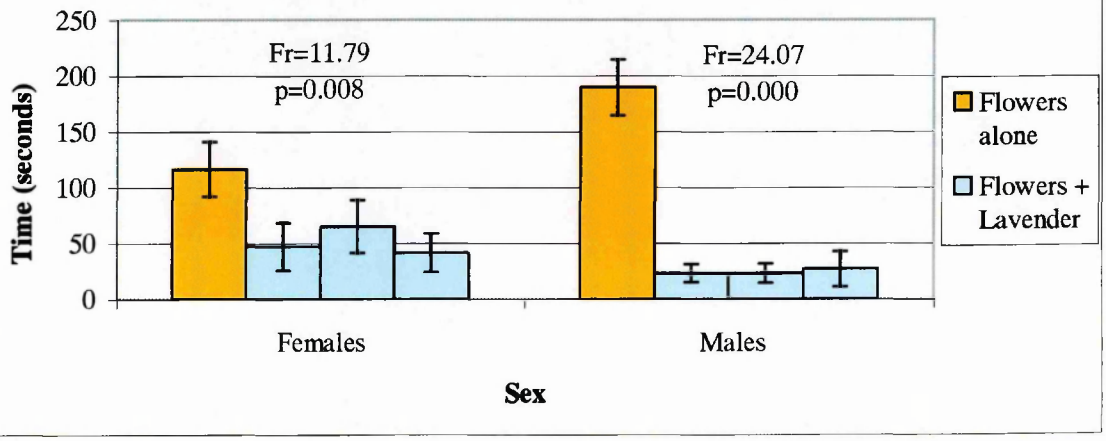


Figure 4.6 Mean number of visits to each arm (±SE)
Experiment 2. Oilseed rape (1 arm) vs oilseed rape + 1% lavender (3 arms)

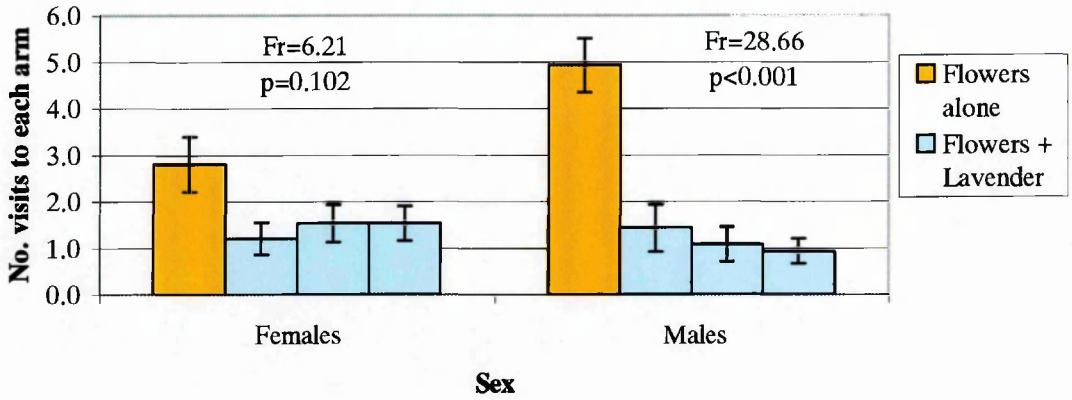


Figure 4.7 Mean time spent in each arm (±SE)
Experiment 3a. Control (1 arm) vs 0.1% Boots lavender (3 arms)

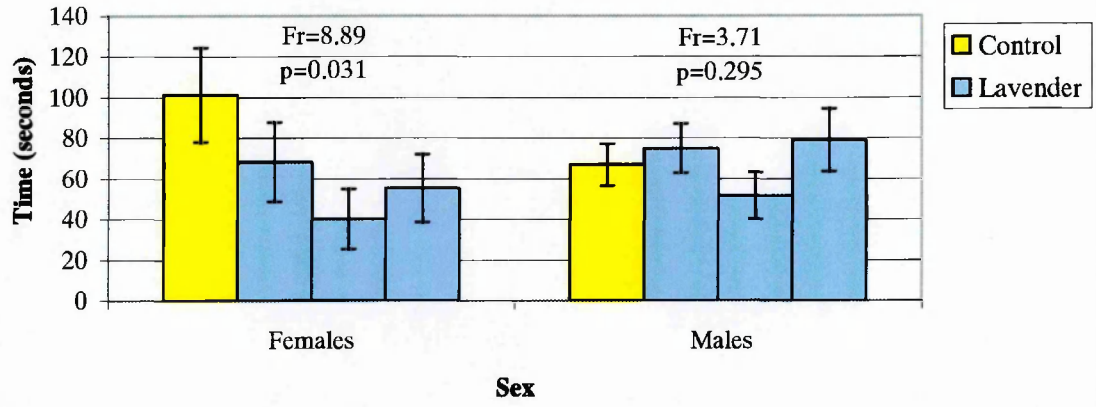


Figure 4.8 Mean number of visits to each arm (±SE)
Experiment 3a. Control (1 arm) vs. 0.1% Boots lavender (3 arms)

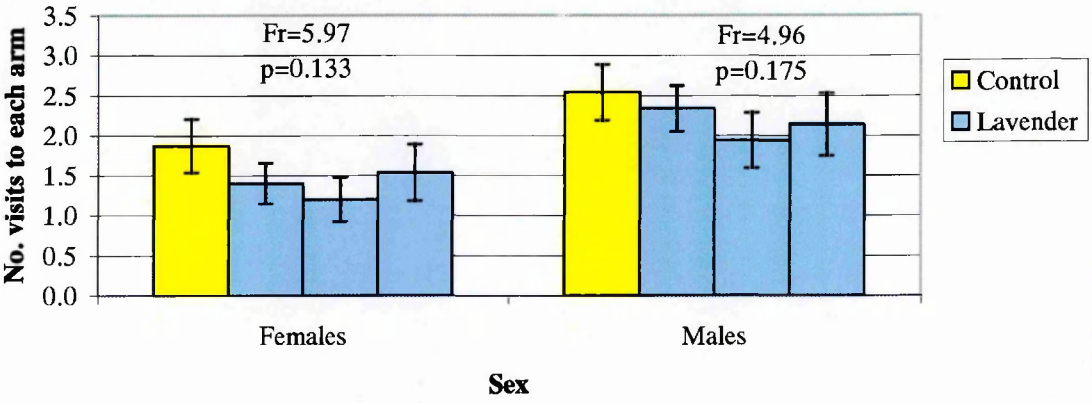


Figure 4.9 Mean time spent in each arm (±SE)
Experiment 3a. Control (1 arm) vs 1% Boots lavender (3 arms)

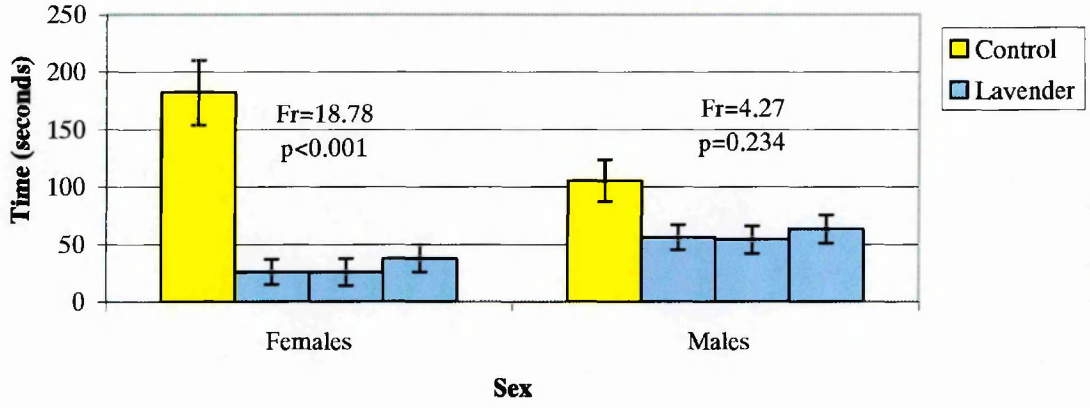


Figure 4.10 Mean number of visits to each arm (±SE)
Experiment 3a. Control (1 arm) vs. 1% Boots lavender (3 arms)

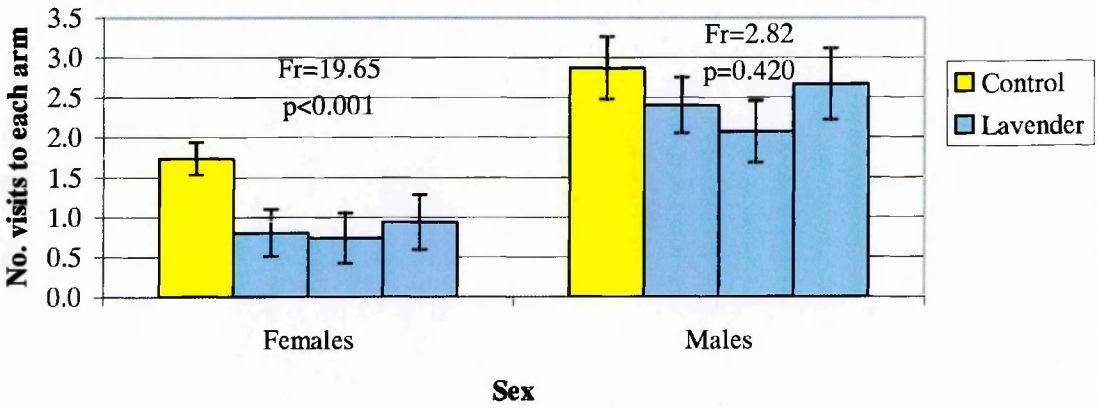


Figure 4.11 Mean time spent in each arm (±SE)
Experiment 3a. Control (1 arm) vs 10% Boots lavender (3 arms)

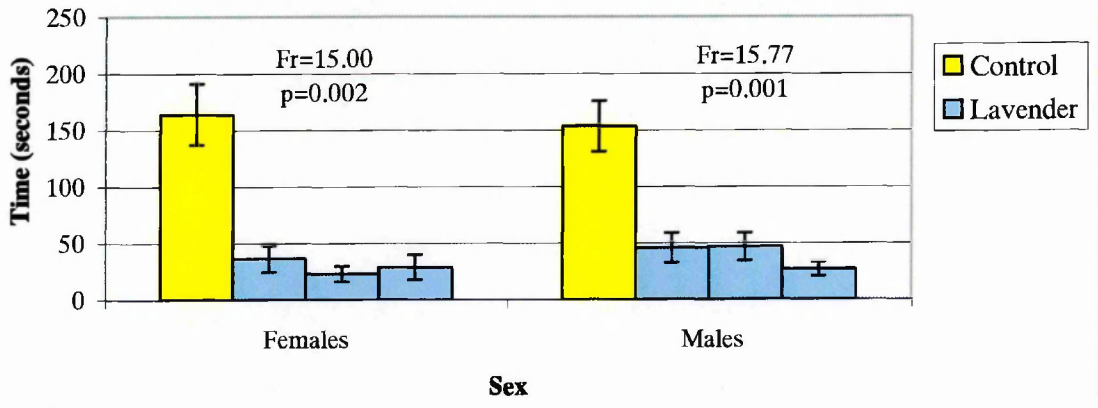


Figure 4.12 Mean number of visits to each arm (±SE)
Experiment 3a. Control (1 arm) vs. 10% Boots lavender (3 arms)

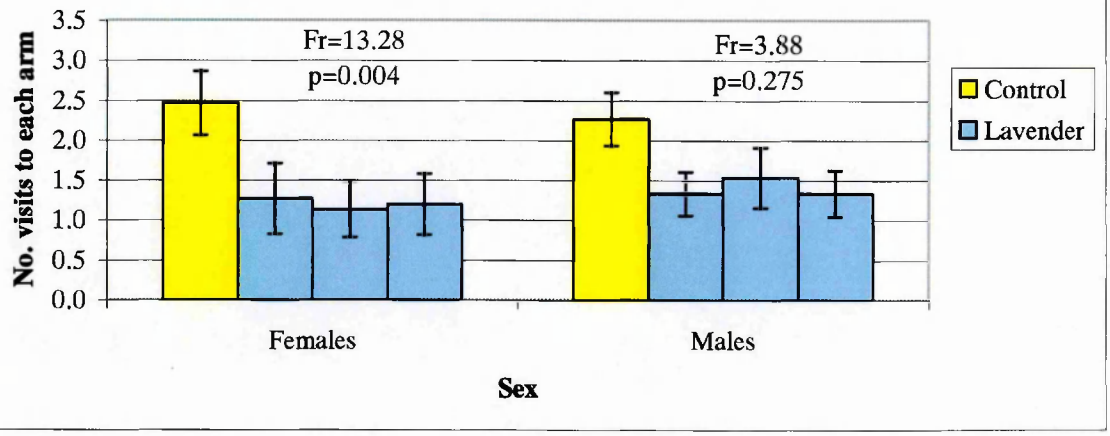


Figure 4.13 Mean time spent in each arm (±SE)
Experiment 3b. Control (1 arm) vs 1% Botanix lavender (3 arms)

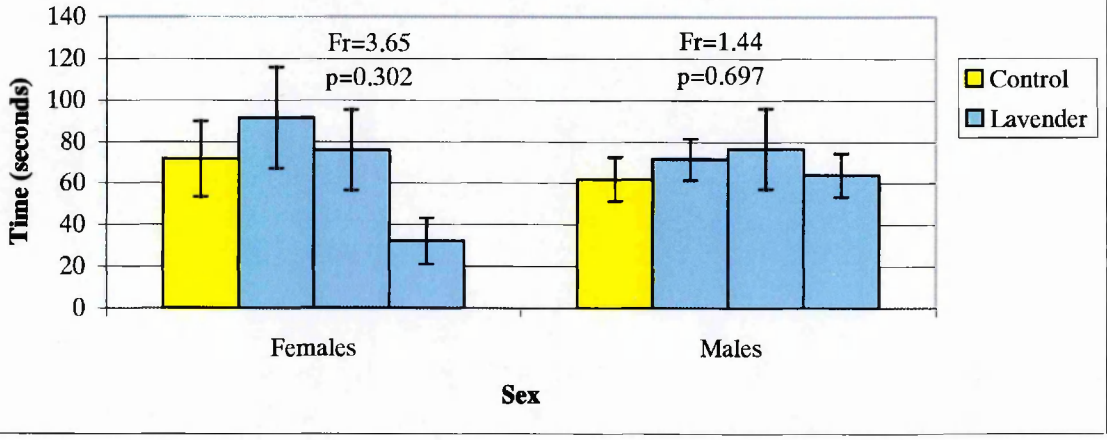


Figure 4.14 Mean number of visits to each arm (±SE)
Experiment 3b. Control (1 arm) vs 1% Botanix lavender (3 arms)

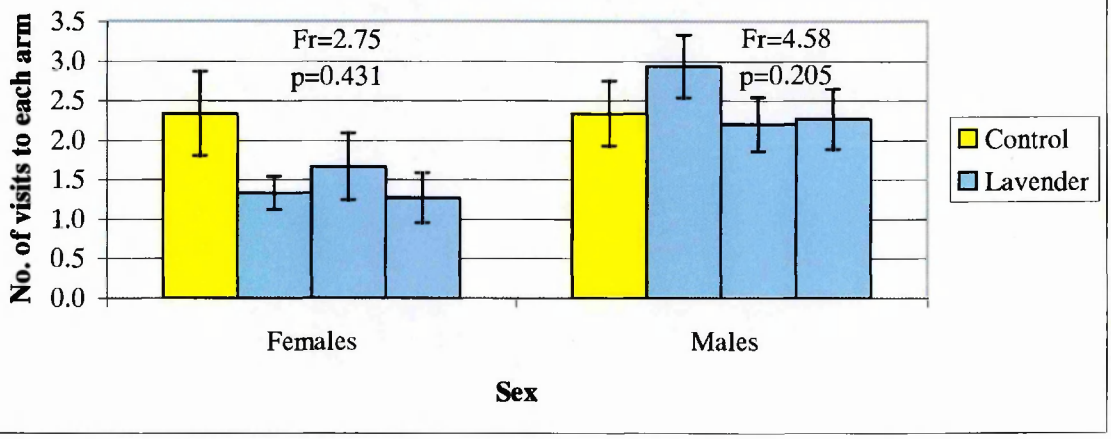


Figure 4.15 Mean time spent in each arm (±SE)
Experiment 3c. Control (1 arm) vs 1% Botanix lavender (3 arms)

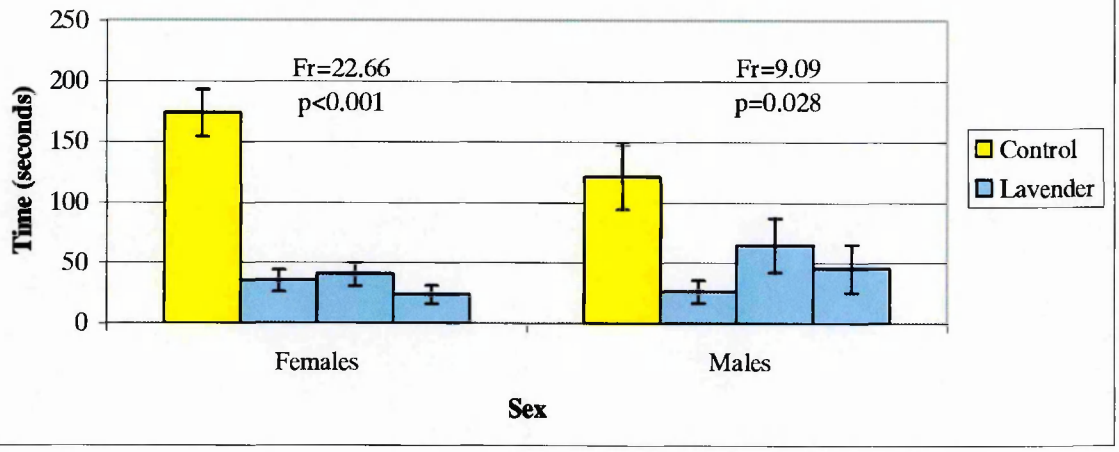


Figure 4.16 Mean number of visits to each arm (±SE)
Experiment 3c. Control (1 arm) vs 1% Botanix lavender (3 arms)

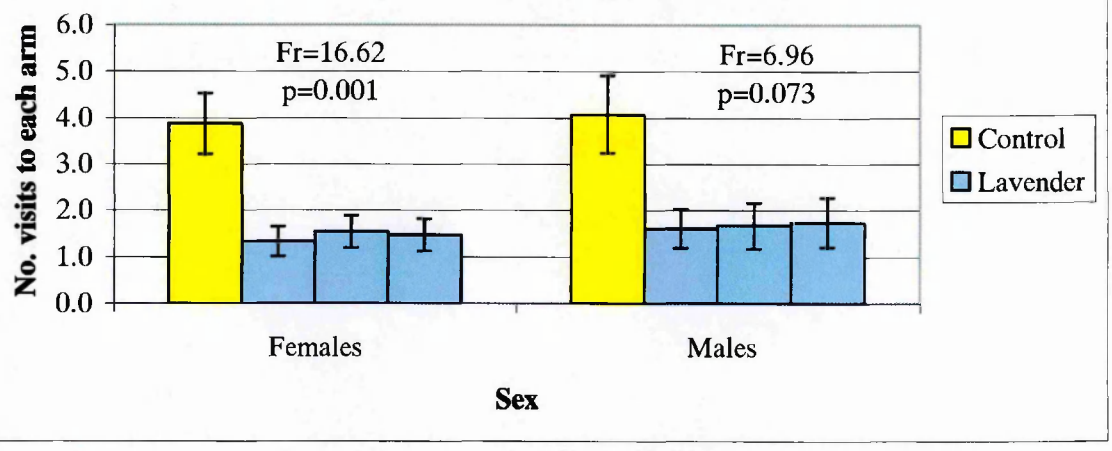


Figure 4.17 Mean time spent in each arm (±SE)
Experiment 4. Control (1 arm) vs 1% Mayweed oil (3 arms)

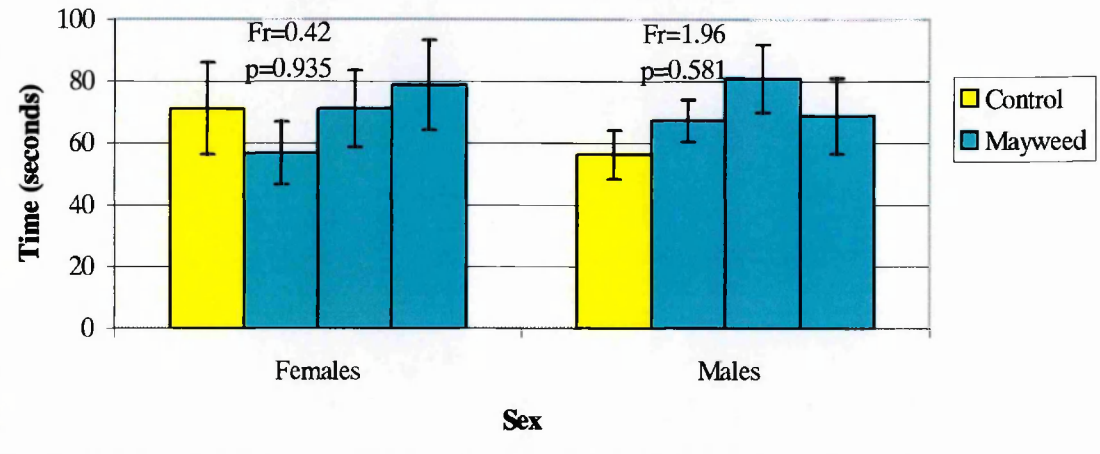


Figure 4.18 Mean number of visits to each arm (±SE)
Experiment 4. Control (1 arm) vs 1% Mayweed oil (3 arms)

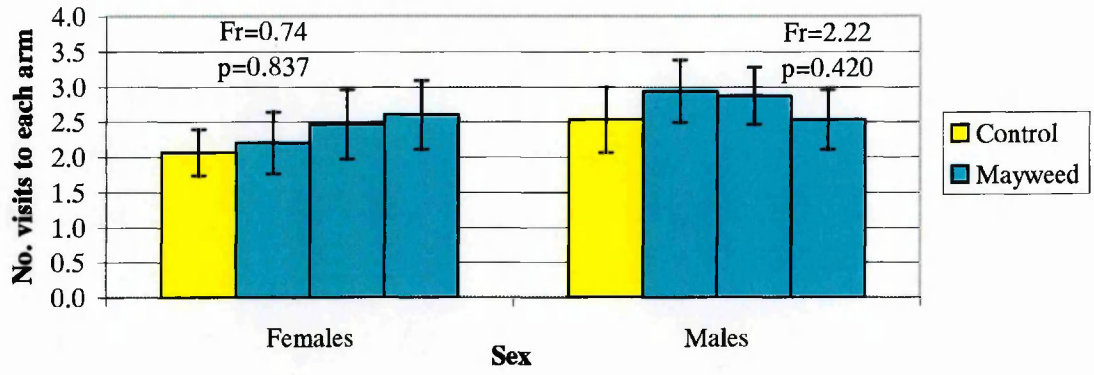


Figure 4.19 Mean time spent in each arm (±SE)
Experiment 5. Control (1 arm) vs 1% Gum haggar (3 arms)

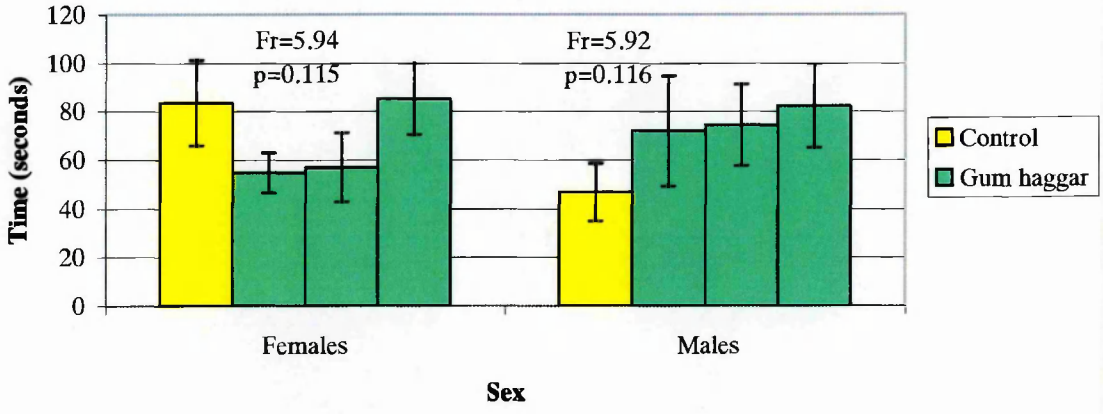
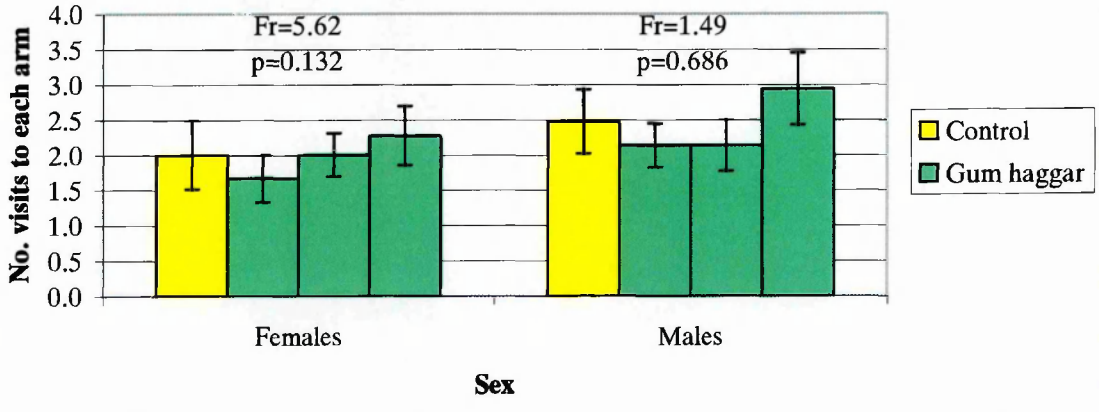


Figure 4.20 Mean number of visits to each arm (±SE)
Experiment 5. Control (1 arm) vs 1% Gum haggar (3 arms)



4.5 DISCUSSION

The 4-arm olfactometer is a very powerful tool for investigating the behavioural responses of insects to volatiles. The presentation of the treatments i.e. three test arms and one control arm, provides better statistical power than 2-way olfactometers such as Y-tubes (Vet *et al.*, 1983). The blank tests showed that the females had a slight preference for arm 4, so in experimental tests, the orientation of the control arm was changed each time, to account for any possible positional preference by the females.

The results from experiment 1 showed, as expected (Ruther & Thiemann, 1997; Blight & Smart, 1999; Smart & Blight, 2000), that both sexes of pollen beetles were attracted to the odour emanating from oilseed rape flowers, even in the absence of visual and contact cues. However, it was important to determine whether the addition of lavender odour was able to over-ride this olfactory attraction. The results from experiment 2 showed that lavender odour did over-ride the attraction and therefore the results from Chapter 3 are likely to be due to the presence of the odour, rather than visual or contact cues. However, experiment 2 does not distinguish between masking and repellency. In order to determine whether lavender odour acts as a repellent in addition to masking host plant attraction, the response of the beetles to the odour alone needs to be tested. If the results in experiment 2 were solely due to reduced attraction to host plant odour (masking) in the presence of lavender odour, then lavender when tested alone would not lead to any avoidance. However, if lavender odour acts as a repellent (in addition to eliminating attraction to host plant odour), then the lavender odour will be avoided by *M. aeneus* when tested alone. This distinction is the subject of experiment 3.

As the results from experiments 3a and 3c show, lavender odour alone elicits avoidance behaviours in *M. aeneus*, proving that it is repellent (as defined on page 7). However, this does not exclude the possibility that the odour may also act to mask attraction to host-plant volatiles. This finding implies that the insects have olfactory receptors capable of detecting volatile components of non-host plants, such as lavender, and that these components can elicit avoidance responses. In order to establish which volatile chemicals are responsible for the repellent effect, techniques such as coupled gas chromatography and electroantennogram studies need to be employed and this line of investigation is the subject of chapter 5.

During experiment 3, there was a large amount of variability in the beetles' responses. Experiments 3a, 3b and 3c all tested responses to 1% lavender essential oil. Experiment 3a used Boots oil and experiments 3b and 3c used Botanix oil, however there was little difference in the volatile profile of the two oils (see chapter 5). Results from experiments 3a and 3c were very similar and the beetles showed strong repellency to the odour, whereas the results from experiment 3b did not show significant differences between the treatments.

The variability might have been due to external meteorological fluctuations affecting the insects' behaviour in the laboratory tests. It is very difficult to assess the effect of the artificial environment on the 'natural' behaviour of the insects. For example, the humidity could have been too low (J. McNeil personal communication). Also barometric pressure fluctuations have been implicated in influencing parasitoid (Steinberg *et al.*, 1992; Marchand & McNeil, 2000) and leafhopper (Anderson *et al.*, 1993) behaviour. No link between barometric pressure and the beetles' responses to lavender could be found in these data (A.L. Mauchline, unpublished data). However, as the weather was very variable during experiment 3b, this could partly explain the lack of response to lavender at this time, however this is just a suggestion, as the other experiments were not tested for pressure effects.

Following this variability in the beetles' responses, repellency to lavender was used as a positive control for further experiments. At the start of each experiment, a few test beetles were tested for an avoidance response to lavender; if those beetles were not responding to the volatiles or remained inactive, the experiments were postponed until the insects were active again.

Such variability is inherent in data from behavioural studies; however, there may be another explanation for this inconsistency, as the timing of the experiments may be important. Cook (2000) identified four distinct lifecycle phases for *M. aeneus* and labelled them as; post-diapause (March-April polyphagous), reproductive (May-June monophagous on Brassicas), pre-diapause (August polyphagous) and diapause (autumn-March) (see table 1.2). Experiment 3b was conducted at the beginning of April using insects recently emerged from overwintering, and it was shown that lavender was not repellent to them. The fact that these post-diapause beetles were unresponsive to lavender might indicate that they were utilising a different range of plant cues to locate their wide range of host plants

during their polyphagous stage and had not yet reached the reproductive stage. Experiment 3c was conducted towards the end of April, at the beginning of the beetles' reproductive phase on Brassicas (Williams & Free, 1978). Lavender repellency was evident during this phase. Experiment 3a was conducted at the end of August, during the pre-diapause phase and repellency of lavender was still present. Therefore, there might be an olfactory mechanism that causes the beetles to remain on oilseed rape while the pollen resource is still available, meaning that non-host plant volatiles become repellent to the beetles on experience of feeding on oilseed rape, and remain so until the food source runs out. In terms of pest control, it is of most importance for the repellent to be effective during the reproductive phase on oilseed rape; therefore these results are encouraging.

This possible explanation is interesting as the results might indicate a host-phase dependent response to plant volatiles. This has been shown to exist in host-alternating aphids. Methyl salicylate is a volatile component of *Prunus padus,* the winter host of the bird-cherry-oat aphid *Rhopalosiphum padi* (L.), and it was found to reduce colonisation of the summer host by this and other cereal aphids (Pettersson *et al.*, 1994). As methyl salicylate is not present among volatiles from the summer host, it may be a cue by which the aphid discriminates between hosts during migration, requiring a modification of its host plant preferences by the aphid itself (Glinwood & Pettersson, 2000a). The principle mode of action of methyl salicylate against the aphids has been shown to be repellency, but the aphid response appears to be dynamic, disappearing after 3-4 days of adult life (Glinwood & Pettersson, 2000b). A similar dynamic response to lavender could be occurring in *M. aeneus* where the switch between polyphagy and monophagy is mediated by a change in response to host plant chemicals and the lavender repellency only becomes evident on the switch to Brassicas during their reproductive period.

Testing for repellency to lavender, as in experiment 3, needs to be repeated throughout the lifecycle of the adults in order to test these speculative explanations, although there was not time during this investigation. Using insects collected at different times through the year from both wild flowers and oilseed rape could test the hypothesis of a dynamic response to lavender throughout the lifecycle of *M. aeneus*.

The importance of concentration was discussed in chapter 3. Again, evidence of a concentration effect was noticeable in the data for experiment 3a. At the highest

concentration of 10% the repellent effect of lavender was strongest. Although the males were no longer repelled by the lowest concentration of 0.1% lavender, the females were repelled and it is most important to control the females in a pest control strategy as they damage the oilseed rape buds by chewing to make oviposition holes.

There appeared to be differences in the reactions of the sexes to the lavender odour in experiment 3a. The females were strongly repelled by the lavender odour and made significantly fewer visits and spent less time in the lavender arms compared to the control arm at all concentrations. However, despite being more active than the females throughout the experiment, the males made a similar number of visits to all the arms and only spent less time in the lavender arms at the highest concentration (10%). This may be because pollen beetles have a wider host range for feeding than for oviposition (Ekbom & Borg, 1996). Females only oviposit on Brassicas and it is important for their larvae that they make the right host choice at oviposition. Although the males are oligophagous at this stage, they still need to locate females to mate. Mating is known to occur on Brassicas from mid-May until the emergence of new generation adults in mid-July (Williams & Free, 1978). Males are therefore expected to be responsive to Brassica odours (results from experiment 1 confirm this), but may also be responsive to other olfactory signals (such as pheromones), so consequently may be less affected by non-host plant odours. This explanation is similar to the conclusions reached by Groot (1999) in a study investigating the sex-related perception of insect and plant volatiles in the green capsid bug *Lygocoris pabulinus.*

It was interesting to compare the responses to lavender essential oil with other essential oils. Pineapple mayweed odour was tested in the olfactometer as it was deemed to be a non-host plant of *M. aeneus* (Appendix 1), however the non-host recognition proved not to be based on olfactory stimuli as no evidence of repellency to the essential oil was seen in experiment 4. The same result was obtained for the *Commiphora erythraea* extract in experiment 5. This is a reminder that a volatile that is strongly biologically active towards certain groups such as aphids (J.A. Pickett, personal communication) and ticks (Carroll *et al.*, 1989) is not necessarily active to another.

Both experiments 1 & 2 were conducted at the end of April/beginning of May 2001 with insects in the early reproductive phase and the results showed that the addition of lavender

odour reduced attraction towards host plant odour. These results are encouraging in terms of using lavender in a pest control strategy as the repellent needs to be able to operate in oilseed rape fields of strong visual and olfactory attraction to pollen beetles.

These experiments have established a protocol for investigating the behavioural responses of *M. aeneus* to odours, and the repellency of lavender can be used as a positive control. However, the host-location behaviour of these insects has not been characterised in detail and the relative importance of olfaction compared to visual and other cues in this process remains to be determined. The main limitation of this methodology is that the insects' responses are observed while walking inside the confined olfactometer chamber. Pollen beetles' natural behaviour is to fly (Williams & Free, 1978; Sedivy & Kocourek, 1994) to their host plants. Therefore, field cage experiments were designed to scale up these laboratory bioassays and investigate the response of *M. aeneus* to non-host plant odours under field conditions. These experiments are described in chapter 6.

CHAPTER 5. INVESTIGATION INTO THE CHEMICAL BASIS FOR RESPONSES OF *MELIGETHES AENEUS* TO NON-HOST PLANT ODOUR

5.1 INTRODUCTION

To understand the olfactory cues associated with the host location behaviour of insects, the detection and response to both the overall odour blend and the individual component compounds need to be investigated. Several techniques are employed, usually starting with behavioural observations, such as those described in Chapters 3 & 4. The use of electrophysiological techniques, combined with analytical chemistry enables identification of the chemicals that are detected by the insect. Behavioural bioassays, for example using the 4-arm olfactometer, are then required to investigate that the behavioural effect of each of the identified chemicals.

5.1.1 Behavioural responses to attractive host plant chemicals

Both male and female *Meligethes aeneus* beetles respond to a wide variety of plant- and insect-derived volatile chemicals. Free and Williams (1978) first established their response to the odour of their host plants using volatile-releasing, yellow water traps. More beetles were caught with extracts of oilseed rape and other crucifers including *Sinapsis arvensis* (wild mustard) and *Alliaria petiola* (garlic mustard) (Free & Williams, 1978). This 'attraction' was due, at least in part, to the characteristic isothiocyanates emitted. Traps baited with allylisothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate or phenylethyl isothiocyanate (amongst others) caught higher numbers of *M. aeneus* than unbaited controls (Free & Williams, 1978; Smart, 1993; Smart *et al.*, 1995; Blight & Smart, 1999). Mixtures of these chemicals were also attractive, but specific combinations were required for maximum attraction (Smart *et al.*, 1995; Smart & Blight, 2000).

Other methods have also been employed to investigate responses of *M. aeneus* to host-plant odours. Mark-release-recapture experiments using traps baited with extracts of rape leaves and flowers were attractive to *M. aeneus* over distances of more than 20 m (Evans & Allen-Williams, 1994). This was also demonstrated in laboratory tests using a 4-arm olfactometer where the oilseed rape was strongly attractive to *M. aeneus* (Evans & Allen-Williams, 1994; chapter 4, this thesis).

Interestingly, not only extracts or volatiles released on insect damage (kairomones) are attractive. Headspace extracted volatiles collected from intact plants in the early bud stage have also been shown to be strongly attractive to *M. aeneus* in Y-tube olfactometer bioassays (Ruther & Thiemann, 1997). Of several plants tested in this study, oilseed rape, tomato and rye were all attractive, but oilseed rape was significantly preferred over the other 5 plants tested, except for tomato. Intact crucifers only release very low concentrations of isothiocyanates, and since these plants were in the early bud stage, floral volatiles were also absent. Therefore, this study provides evidence that *M. aeneus* is also attracted to rape green leaf volatiles. However, the greatest attraction of *M. aeneus* was achieved using a complex mixture of both general green leaf plant volatiles and host-specific chemicals (Smart *et al.*, 1995; Smart & Blight, 2000).

5.1.2 Behavioural responses to plant chemicals

Not all plant odours are attractive to *M. aeneus*. Some plant chemicals are repellent; even some host-plant volatiles have repellent effects when tested alone. Of the chemicals extracted from Brassicaceous plants and tested in the field using water traps, 15 were attractive to *M. aeneus*, five were inactive, but five reduced the number of beetles caught (Smart & Blight, 2000). This finding reinforces the importance of the blend of volatiles in eliciting different behaviours in *M. aeneus*. The repellents included 1-octen-3-ol, 1-hexanol, (Z)-3-hexen-1-ol, 1-pentanol and *cis*-jasmone. Many of these had variable effects depending on the concentration used. However, 1-octen-3-ol was found to be repellent at all concentrations tested.

5.1.3 Behavioural responses to insect-derived volatiles

Insect-derived volatiles are also important in the ecology of *M. aeneus*. There is evidence that female beetles (but not males) actively avoid conspecifics, suggesting the release of an epideictic or spacing pheromone (Ruther & Thiemann, 1997). Insect-derived repellents provide another possible source of volatiles for use in pest control, but this study is only concerned with plant-derived volatiles.

5.1.4 Analytical chemistry techniques

Gas chromatography (GC) can be used to separate the chemicals found in a complex volatile blend (McNair & Bonelli, 1969), for example in essential oils (Adams, 1995), headspace extractions (Tollsten & Bergstrom, 1988) and insect pheromone extracts (Wadhams, 1990). Gas chromatography is an analytical technique whereby volatile samples are separated on a GC column on the basis of compounds' mobility in a mobile gas phase and their interaction with a bound stationary phase. Samples are introduced via an injector port, and, after passing through the column, the separated peaks are detected, usually by burning in a flame ionization detector (FID). The nature of the GC stationary phase and the GC oven temperature programme determine retention time for each individual peak and were selected to give standard results.

Gas chromatography cannot provide chemical identities for the compounds; this requires a technique such as mass spectrometry (MS). Analysis by MS involves samples being introduced into a chamber (the "source") under high temperature and vacuum, and fragmenting under bombardment by small particles, typically electrons (Ashcroft, 1997). The fragments (including positively charged ions) pass from the source into an analyser, which separates the fragments by passing through a scanning magnet. The charged ions then reach a detector, which records the fragmentation pattern. Analysis of the pattern, and comparison with libraries can lead to the tentative identification of compounds, as each chemical provides a characteristic fragmentation pattern. In coupled GC-MS each peak in a GC trace can be tentatively identified (Williams, 1996).

5.1.5 Recording insect olfaction

Insects detect volatile odours mostly via olfactory receptors on their antennae (Visser, 1986). The olfactory receptors are modified sensillae with porous hair shafts (Bartlet *et al.*, 1999a) that are designed to detect airborne volatiles. At the base of the sensillae, dendritic membranes of sensory cells have 'acceptors' for the odour molecules. When these odour molecules bind to the acceptors, the membrane conductance is changed resulting in a decrease in the resting potential (Visser, 1986). The generated nerve impulses are conducted to the central nervous system and these signals can be measured in two ways. Firstly, single cell recordings can identify responses from individual neurones (Den Otter *et al.*, 1980; Tommeras & Mustaparta, 1989). Secondly, the use of an electroantennogram (EAG) can record the electrical response from all the sensilla on the antenna (Blackwell *et*

al., 1997; Groot *et al.*, 1999). In this way, volatile chemicals can be individually tested to determine whether the insect can detect them.

To locate antennal responses to individual compounds within a complex volatile blend, GC can be coupled to electroantennography. As peaks are separated on the GC column, the effluent is split between the FID and a stream of humidified, purified air, which is positioned over an insect antenna to which tungsten microelectrodes are inserted. If a compound is detected by any receptors on the antenna, an electrical impulse is recorded. Comparison of the GC and EAG traces can locate individual compounds that are perceived by antennal receptors from within a complex blend. The active compounds can then be chemically identified by trace comparison with mass spectrometry analysis (Blight *et al.*, 1995c).

5.1.6 Linking chemistry and behaviour

Results obtained from electrophysiological studies of the receptor systems of insects can only be understood in the context of the insect-host relationship, when combined with behavioural studies (Schoonhoven, 1968). Therefore, each chemical detected by the receptors of an insect can potentially have one of several effects on its behaviour. Chemicals can cause movements towards (attractant) or away from the source (repellent) and can elicit (stimulant) or inhibit (deterrent) feeding, mating or oviposition. Alternatively, chemicals can stimulate locomotion (without repellency), act as an arrestant (slowing locomotion leading to aggregation) or even have a neutral effect on behaviour (Dethier *et al.*, 1960). The effects vary amongst organisms and their physiological states, as well as varying between different concentrations of the chemical. Therefore, the conditions under which these terms are employed should be specified.

5.1.7 Olfactory detection of volatiles by *M. aeneus*

There have been few studies investigating the olfactory detection of volatiles by *M. aeneus* despite the range of laboratory and field demonstrations of behavioural responses to plant volatiles (Sections 5.1.1 & 5.1.2). The one such study of *M. aeneus* used coupled GC-EAG to locate compounds in a mixture of oilseed rape volatiles, which are detected by their antennae. Twenty-six compounds elicited antennal responses and these were produced after exposure to volatiles extracted from intact flowering shoots and leaves of oilseed

rape. From the volatiles eliciting EAG responses, three isothiocyanates were identified (Blight *et al.*, 1995b).

Lavender essential oil contains approximately 30 readily detectable, volatile compounds (Williams, 1996). As the results from chapters 3&4 show, the oil is repellent to *M. aeneus*. It is likely that some of the volatiles will be repellent, whilst some may be attractive and many are likely to be of no behavioural relevance. Specific ratios of important compounds could also be responsible for the repellency of the non-host odour (Blight *et al.*, 1995c; Al Abassi *et al.*, 2000). It is important to establish the biologically active compounds within the overall lavender blend and therefore, using the electrophysiological and analytical techniques described, this forms the basis of this chapter.

5.2 AIMS

1. To quantify and identify the volatile chemicals within lavender essential oil.
2. To establish which chemicals within lavender essential oil are detectable by antennal receptors of *M. aeneus*.
3. To determine the behavioural responses of *M. aeneus* to these compounds.

5.3 TECHNIQUES

5.3.1 Gas chromatography (GC)

Samples were analysed using a Hewlett-Packard 5890A gas chromatograph (GC) equipped with a cool on-column injector, a non-polar HP-1 bonded phase fused silica capillary column (50 m x 0.32 mm i.d. x 2.65 μm film thickness), and a flame ionisation detector (FID). The oven temperature was maintained at 40°C for 1 minute, then 5 °C/minute to 150 °C, held at 150 °C for 0.1 minute and 10 °C/minute to 250 °C. The carrier gas was hydrogen.

5.3.2 GC-Mass spectrometry (GC-MS)

GC-MS analysis was conducted by Dr M.A. Birkett, Biological Chemsitry Division, Rothamsted. A capillary GC column (50 m x 0.32 mm i.d. HP-1) fitted with a cool on-column injector was directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments, Manchester, UK). Ionisation was by electron impact at 70eV, 250°C. The

oven temperature was maintained at 30°C for 5 minutes and then programmed at 5°/minute to 250°C.

5.3.3 Electroantennography (EAG)

Electroantennogram (EAG) recordings were made using Ag-AgCl glass electrodes filled with saline solution:

Composition of Insect Ringer solution (in 1 litre)	
Sodium chloride	7.55g
Potassium chloride	0.64g
Calcium chloride (dihydrate)	0.22g
Magnesium chloride	1.73g
Sodium bicarbonate	0.86g
Sodium orthophosphate	0.61g

The glass electrodes were made from borosilicate, thin wall, glass capillaries without filaments (1.5 mm outside diameter, 1.17 mm internal diameter). The electrodes were formed by heating a capillary under tension until the glass was pulled apart into two fine points. The tip of the electrode was then cut by hand to produce the correct-sized hole to hold the antenna.

Meligethes aeneus were field collected and kept in culture (Section 2.2). Beetles were sexed and starved overnight prior to testing. An adult pollen beetle was anaesthetised by chilling on ice while an antenna was excised using a scalpel and then the basal end of the antenna was inserted into the indifferent electrode. The electrode was fitted into a holder and the recording electrode, already secured in its holder, was brought forward to contact the distal end of the antenna (Figure 5.1). Signals from the antenna were amplified (x10,000) and analysed using a customised software package (Syntech, The Netherlands).

Initial response of the antenna to the sample was established using the stimulus delivery device (Syntech, The Netherlands) (Figure 5.1). Lavender essential oil (Botanix) (10 µl of oil in 1.5 ml redistilled hexane) was applied to a strip of filter paper and the hexane was allowed to evaporate before the strips were placed in a Pasteur pipette. The lavender stimulus (2-second duration) was delivered into a purified airstream (1 litre/min) flowing

continuously over the antenna. The antenna was discarded if the response to lavender was small or if the trace was very noisy.

The response from the antenna was recorded using Electro AntennoGraphy software (Syntech, The Netherlands). When the signal baseline had settled and a significant response was elicited from the lavender puff, the antenna was coupled to gas chromatography and both outputs were monitored using Electro Antenno Detection (Syntech, The Netherlands) software package.

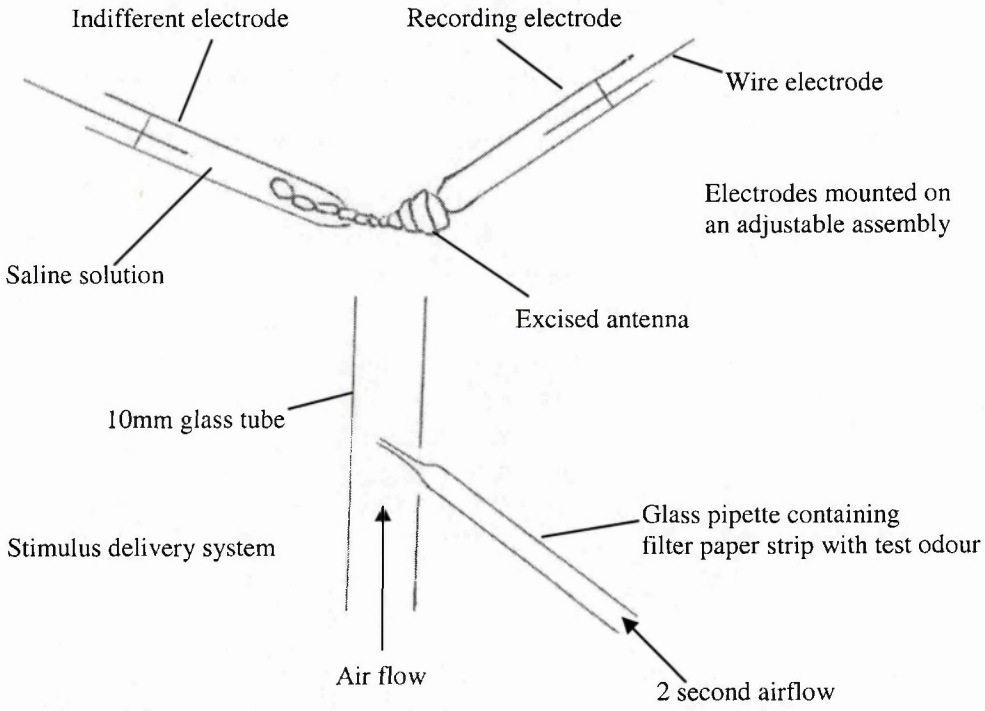


Figure 5.1 EAG assembly and stimulus delivery system

5.3.4 Weight of lavender oil

100 µl of Botanix lavender oil was weighed on a five-point balance as 82.19 mg. This was used to calculate the weight to volume (w:v) concentrations of the samples.

5.4 MATERIALS, METHODS AND RESULTS

5.4.1 Comparison of Boots and Botanix lavender oils

5.4.1.1 Methods

Lavender essential oil from both suppliers (Boots and Botanix Ltd) was analysed by GC to identify any differences in composition. The Boots lavender oil was used for experiments in chapters 3 & 4. The Botanix lavender oil was used for experiments in chapters 4, 6 &7. Samples of each oil were prepared by diluting 2 µl of essential oil in 1.5 ml redistilled hexane (~1 mg/ml). 1 µl of each sample was injected onto the column (section 5.3.1) and the traces compared.

5.4.1.2 Results

The GC traces for lavender essential oil from the two sources were very similar (Figures 5.2 and 5.3). There were a few differences in the minor components, but the ratios of the two main peaks were slightly different. The two main compounds are linalool (the first large peak) and its derivative linalyl acetate (the second large peak) (Williams, 1996). The fact that the Boots oil has more linalyl acetate compared to the linalool may indicate that the linalool has been subjected to degradation by exposure to the air. Therefore, it was decided that the remaining experiments in this thesis would use the Botanix oil only, as it could be repeatedly obtained from a consistent source with reliable storage methods.

5.4.2 Investigation of antennal responses of *M. aeneus* to lavender odour using coupled GC-EAG

5.4.2.1 Methods

A standard 1 mV signal was applied to a test antenna and the experimental responses to the puff of lavender odour were calibrated to this standard. 20 female and 8 male antennae were tested. From the 28 antennal preparations, 5 were also coupled to the GC (Figure 5.4).

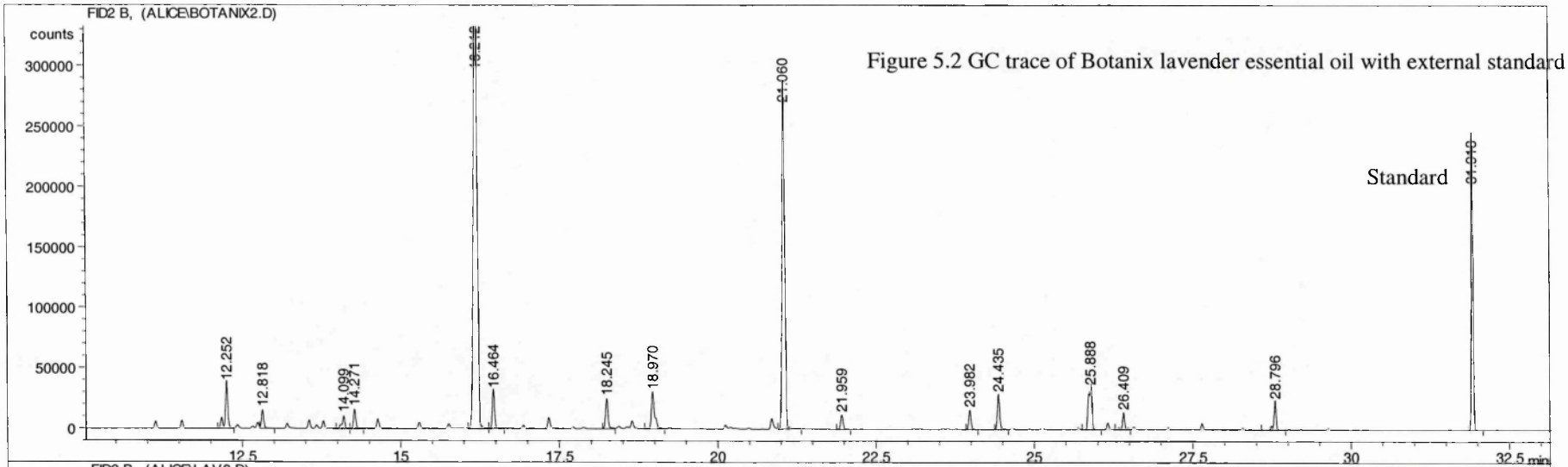


Figure 5.2 GC trace of Botanix lavender essential oil with external standard

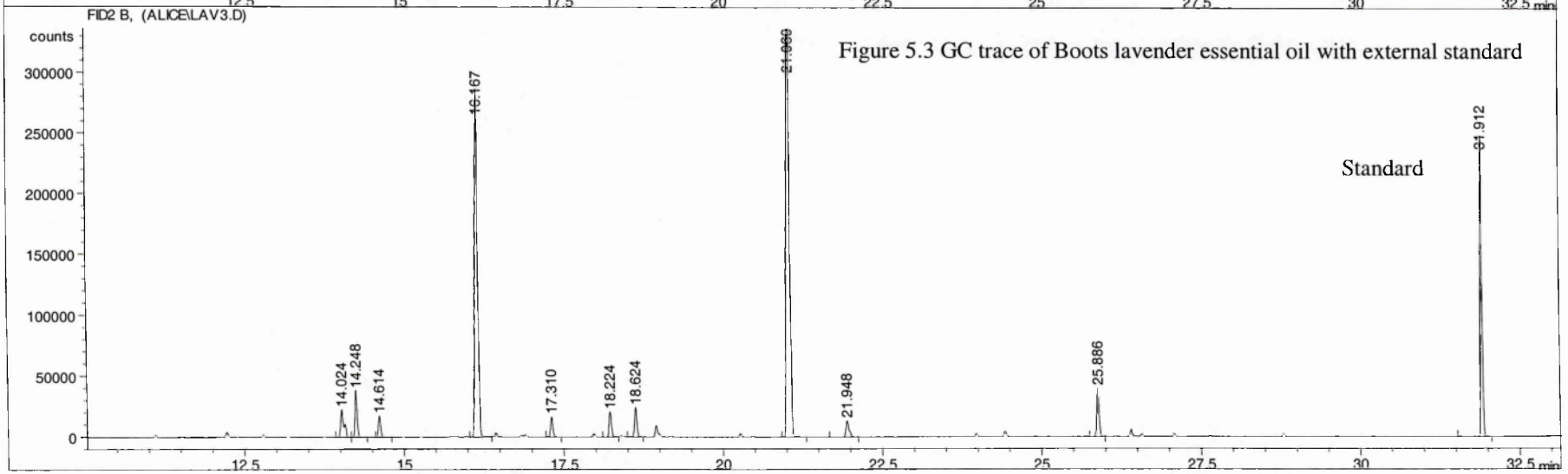


Figure 5.3 GC trace of Boots lavender essential oil with external standard

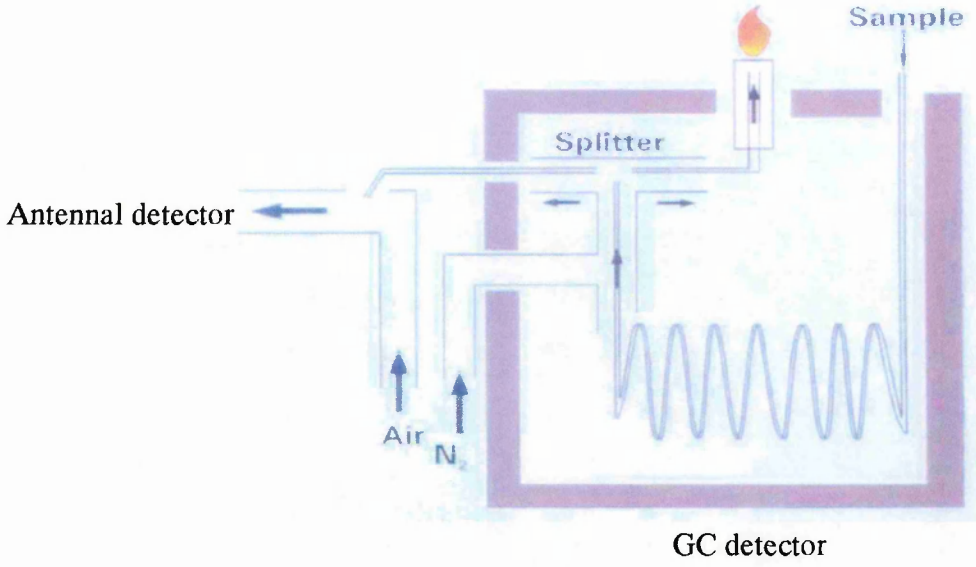


Figure 5.4 Coupled GC-EAG recording system (with permission from C.M. Woodcock)

5.4.2.2 Results

Antennae from both sexes of *M. aeneus* showed strong electrical responses to puffs of lavender odour. The mean response from 20 female antennae was 1.35 mV (±0.22). The mean response from 8 male antennae was 1.25 mV (±0.33). Figure 5.5 shows a trace from a female antenna on stimulation from a puff of lavender volatiles.

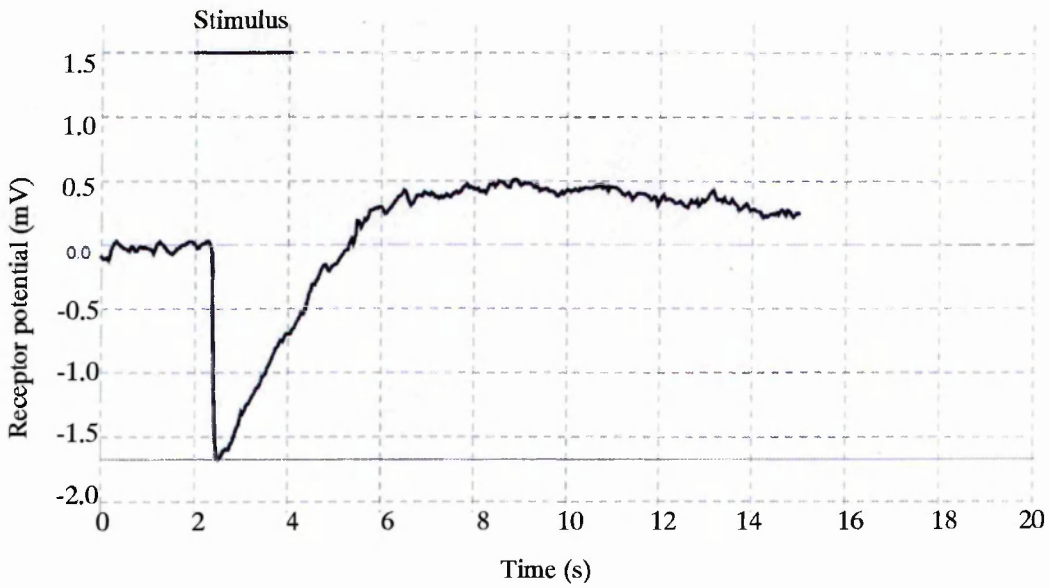


Figure 5.5 EAG trace (showing change in receptor potential) from a female pollen beetle antenna on stimulation from a sample of lavender oil

The traces from 5 coupled runs were compared to pick out consistent EAG activity to individual chemicals. Approximately 15 compounds elicited consistent activity on the EAG and are marked on a composite GC trace illustrated with a sample EAG trace (Figure 5.6).

5.4.3 GC-MS identification of lavender constituents

5.4.3.1 Methods

Botanix lavender oil (1 mg/ml) was analysed by GC-MS to chemically identify the constituents of the oil. This provided a list of tentative identifications of the chemicals.

5.4.3.2 Results

The tentative identifications for the major peaks on the EAG-GC trace for lavender essential oil are shown in Table 5.1. Figure 5.7 shows these chemicals labelled on a GC-MS trace of lavender oil.

Table 5.1 List of constituents of lavender essential oil from GC-MS identification

Peak number	Compound
350	3-octanone
366	myrcene
377	hexyl acetate
456	linalool
521	(+)-terpinen-4-ol
581	linalyl acetate
713	α-santalene
715	β-caryophyllene

5.4.4 Comparison of the GC-MS and coupled GC-EAG data

5.4.4.1 Methods

The traces from the GC-MS and coupled GC-EAG experiments were aligned using comparisons with standard C_8-C_{24} alkane series that were run on both machines at the time of the experimentation. The traces were compared so that the GC peaks with corresponding EAG responses could be matched with the chemical identifications from the GC-MS results.

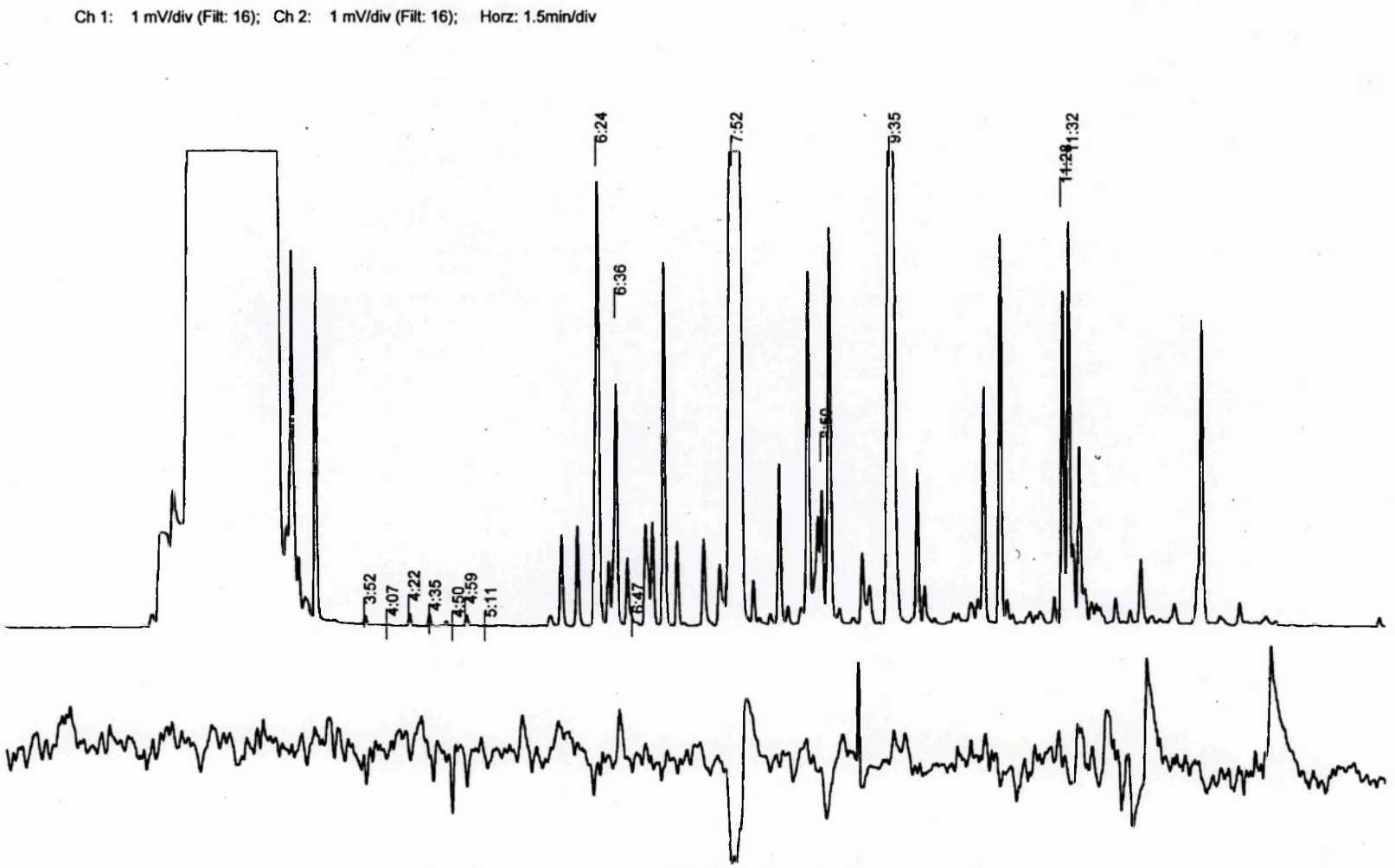
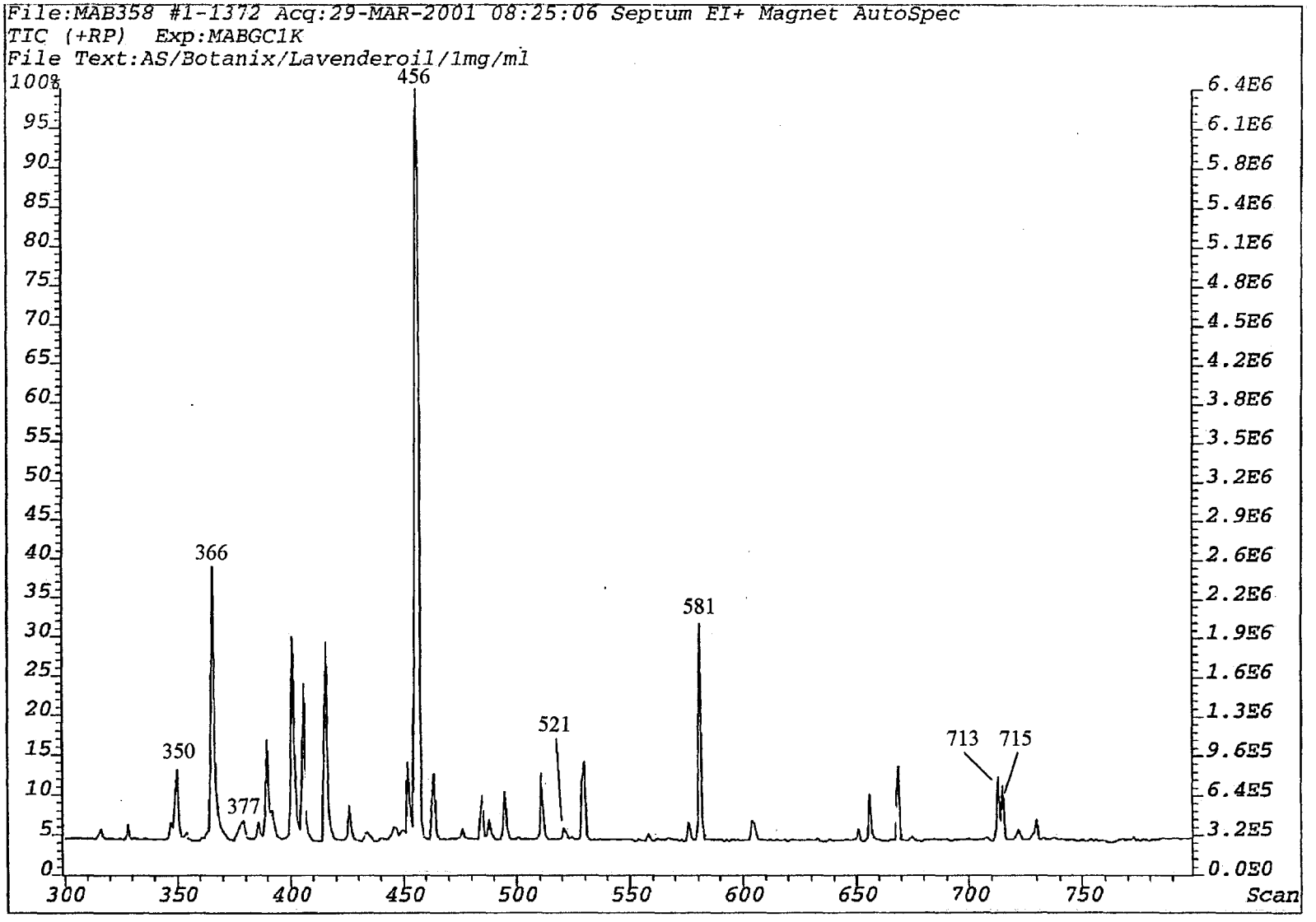


Figure 5.6 Composite GC trace illustrated with an EAG underneath. Times (min) indicate GC peaks with consistent EAG responses from 5 coupled runs.

Figure 5.7 GC-MS trace of lavender essential oil with the EAG active peaks labelled



5.4.4.2 Results

8 of the 15 chemicals located using coupled GC-EAG were tentatively identified by GC-MS. The first 7 EAG active peaks were present in small quantities, which are difficult to identify. Comparison of the GC-EAG and GC-MS traces enabled tentative identifications of the large active peaks (Table 5.1). Chemical structures are shown in Figure 5.8.

5.4.5 Confirmation of chemical identity of EAG active peaks by GC peak enhancement

5.4.5.1 Methods

The tentative identifications, provided by the GC-MS data, for the peaks that were active in the coupled GC-EAG trace were confirmed by co-injections of authentic samples with a sample of lavender oil on two different GC columns, HP-1 (non-polar) and HP-wax (polar). Enhancement of the tentatively identified peak on both columns signified correct identification.

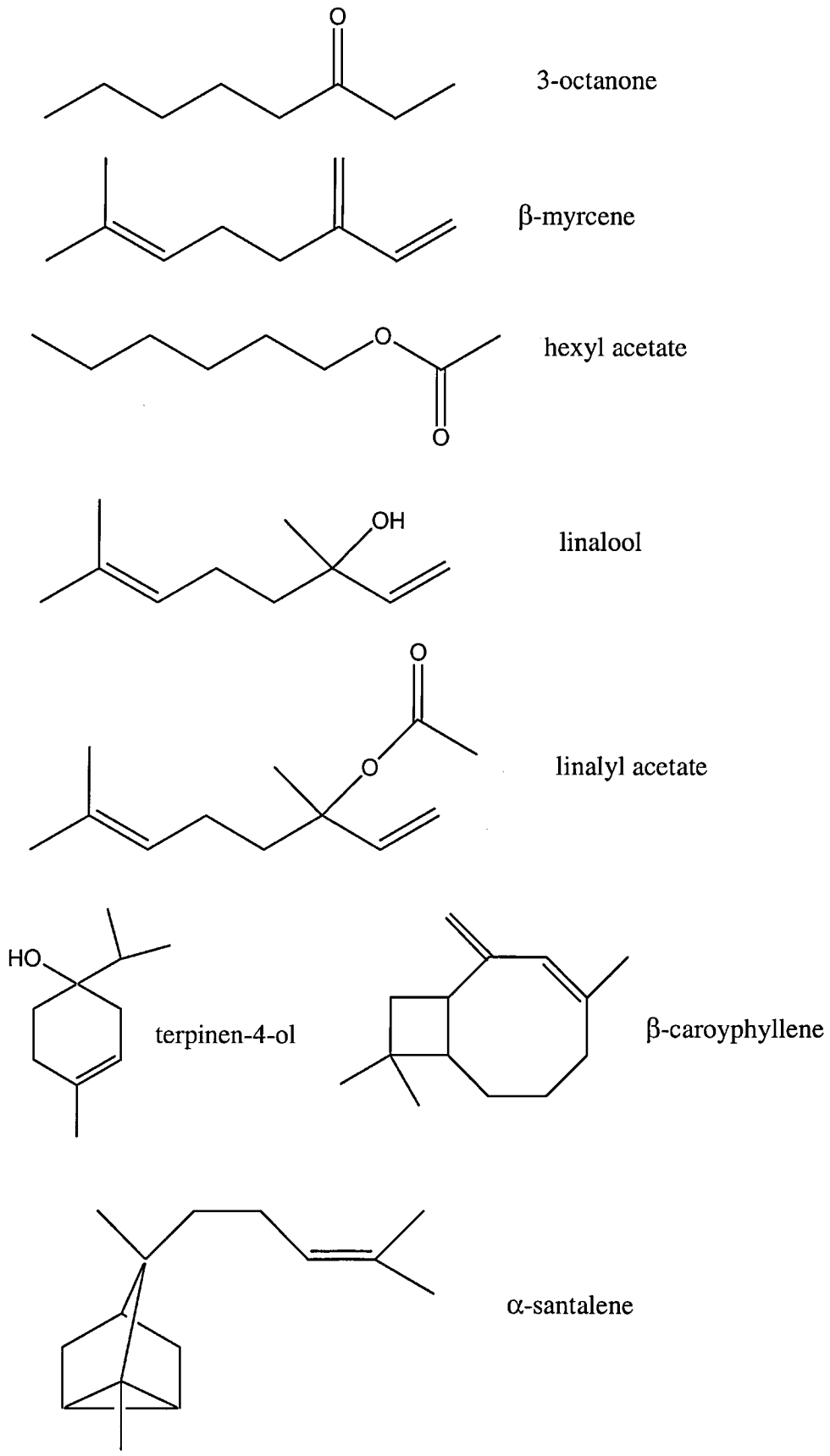
Table 5.2 Co-injection volumes used for GC peak enhancement

Chemical	Source	Concentration	Co-injection volume
3-octanone	Aldrich	100 ng/µl	0.1 µl
myrcene	Fluka	100 ng/µl	0.1 µl
hexyl acetate	Aldrich	100 ng/µl	0.1 µl
linalool	Avocado	100 ng/µl	0.5 µl
(+)-terpinen-4-ol	Fluka	100 ng/µl	0.1 µl
linalyl acetate	Fluka	100 ng/µl	0.3 µl
β-caryophyllene	Koch-Light Laboratories	100 ng/µl	0.1 µl

The above chemicals were prepared and tested in the following ways;

1. 1 µl of chemical (100 ng/µl) injected simultaneously on to HP-1 and HP-wax columns
2. 1 µl of Botanix lavender essential oil (100 ng/µl) and variable volumes of the chemical (100 ng/µl) injected simultaneously on to HP-1 and HP-wax columns - see Table 5.2 for the volume of chemical injected. The volumes were chosen to approximately double the size of the appropriate peak on the lavender trace.

Figure 5.8 Chemical structures of the lavender oil volatiles detected by the antennae of *M. aeneus*



5.4.5.2 Results

The 7 chemicals tested by co-injection peak enhancement on the GC were correctly identified and peak enhancements on the specific parts of the trace were achieved without any increase in peak width. The final active peak on the GC-EAG trace was tentatively identified as α-santalene, however this could not be confirmed, as it is not commercially available.

5.4.6 Quantification of the chemicals within lavender oil

5.4.6.1 Methods

A C_8-C_{24} alkane series was run on the GC under the same conditions as the lavender sample. n-Octadecane was identified as an appropriate standard as it peaks on a flat part of the lavender oil GC trace.

GC co-injections were performed using the chemicals identified in section 5.4.3.2 and n-octadecane as an internal standard. For each chemical, a 1:1 mixture with n-octadecane was prepared (both at 100ng/μl). Dual injections of 2μl of this mixture were conducted using the HP-1 and HP-wax columns simultaneously. The ratio of the HP-1 GC peak areas for the chemicals to the standard (n-octadecane) were compared to provide the response factors. The response factors were then used to calculate the concentration of each chemical making up lavender oil from the lavender/n-octadecane co-injection (see linalool example below).

Example - Linalool

Chemical GC injections;

100 ng Linalool area counts = 2067

100 ng octadecane area counts = 2002

Response factor (RF) = 2067/2002 = 1.032

Lavender GC injection;

100 ng n-Octadecane area counts = 1595

x ng linalool area counts = 568

Therefore, if linalool was present in lavender at 100% (i.e. 100 ng) it should be the same as the standard (100 ng of octadecane) i.e. 1595 x RF = 1595 x 1.032 = 1646. However, linalool only makes up 568 counts (pA) within the lavender.

So, linalool constitutes 568/1646 x 100 = 34.51% of lavender oil (i.e. there are 34 ng in every 100 ng of lavender oil).

When testing lavender at 1% v/v or 8.2 mg/ml, the linalool was at a concentration of 35% of 8.2 mg/ml = 2.87 mg/ml.

5.4.6.2 Results

The response factors of the chemicals were calculated as described in section 5.3.2.6. Table 5.3 shows for each chemical the percentage of the total volume of lavender oil and the concentration (mg/ml) of each chemical in 1% oil.

Table 5.3 Concentrations of chemicals within 1% lavender oil

Chemical	Percent of lavender oil (%)	Concentration in 1% oil (mg/ml)
3-octanone	2.62	0.215
myrcene	0.679	0.056
hexyl acetate	0.274	0.022
linalool	34.51	2.87
(+)-terpinen-4-ol	0.299	0.025
linalyl acetate	18.68	1.56
β-caryophyllene	3.328	0.273

5.4.7 Preparation of chemicals for behavioural testing

1 % lavender oil was taken as the standard concentration where a repellent response in *M. aeneus* is detectable (Chapters 3 &4). 1% lavender oil is 8.2 mg/ml, so the concentration of each of the 7 identified chemicals within 1 % lavender was calculated by taking a percentage of this concentration (section 5.4.6). These solutions were prepared in acetone and used for behavioural experiments. See Table 5.4 for the concentrations;

Table 5.4 Concentrations of chemicals within 1 % lavender (8.2 mg/ml) used for behavioural testing

Chemical	Concentration
3-octanone	0.2 mg/ml
myrcene	0.05 mg/ml
hexyl acetate	0.02 mg/ml
linalool	3 mg/ml
(+)-terpinen-4-ol	25 µg/ml
linalyl acetate	1.5 mg/ml
β-caryophyllene	0.3 mg/ml

The solutions listed in Table 5.4 were prepared in acetone and stored at 5°C until used in the behavioural bioassays.

5.4.8 Testing of the chemicals for behavioural responses of *M. aeneus*

5.4.8.1 Methods

A 4-way olfactometer was used to test the responses of *M. aeneus* to the chemicals identified in this chapter. The standard protocol is detailed in section 4.3.3. The 7 chemicals were prepared at the concentration at which they occur within 1 % lavender oil, as described in section 5.3.2.7. Each chemical was tested for repellency against 15 female *M. aeneus* by presenting 10 µl of the chemical through three arms of the olfactometer and leaving one arm blank. The data were analysed using Friedman's non-parametric ANOVA (Siegel & Castellan, 1988).

5.4.8.2 Results

The results from testing female *M. aeneus* in the four-arm olfactometer are shown in Table 5.5. The mean time spent in the control arms compared to the treated arm is shown. For

each chemical n=15 with 3 degrees of freedom. The two chemicals that were shown to be significantly repellent were linalool (p=0.028) and linalyl acetate (p=0.002). The rest of the chemicals were not significantly avoided. Despite not being significantly different (p=0.118), the mean time spent in the control arm (129 seconds) was much higher than the mean time spent in the arms treated with 3-octanone (50 seconds).

Table 5.5 Mean time spent in control and treated arms of the olfactometer

Chemical	Control mean (s) ± SE	Treatment mean (s) ± SE	Friedman's statistic (adjusted for ties)	p-value
3-octanone	128.5 ± 29.2	49.6 ± 17.1	5.9	0.118
myrcene	106.2 ± 23.4	59.6 ± 16.2	1.9	0.581
hexyl acetate	71.4 ± 21.8	69.7 ± 17.0	1.6	0.655
linalool	161.2 ± 28.7	37.6 ± 12.6	9.1	0.028
(+)-terpinen-4-ol	63.7 ± 19.9	63.8 ± 20.3	2.4	0.488
linalyl acetate	120.3 ± 17.7	52.8 ± 14.6	15.1	0.002
β-caryophyllene	64.5 ± 10.6	72.4 ± 14.7	1.1	0.775

5.5 DISCUSSION

Previous scientific investigations into the volatile chemicals detected by pollen beetles have mainly been focused on attractants. Volatiles from the host plant *Brassica napus* were tested individually and most were attractants, although there were some that had a repellent effect (Smart & Blight, 2000). The repellents were mainly volatile fatty acid derivatives including 1-octen-3-ol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-pentanol and *cis*-jasmone. Some are known to have similar repellent effects in other biological systems. For example, *cis*-jasmone release is induced by insect damage and acts as an aphid repellent while also attracting aphid natural enemies such as ladybirds and parasitoids (Birkett *et al.*, 2000). These repellent chemicals are mainly secondary volatiles, produced by various enzymatic effects on damage to the plant (Schreier, 1984). These could provide a route for genetic engineering or plant breeding of oilseed rape to produce a variety that has high release rates of these repellent chemicals. This would complement the possibility of reducing the emission of glucosinolate metabolites by rape plants, using the same techniques, to make it more difficult for rape pests to orientate to the crop (Bartlet *et al.*, 1999b). However, reduction of the defensive compounds may lead to higher colonisation rates of generalist herbivores (Milford *et al.*, 1989).

The research detailed in this chapter shows a different application for plant-derived repellent compounds. In particular, the use of non-host plant derived repellents provides a method for repelling pest insects without the need for plant breeding or genetic manipulation, while enabling the oilseed rape plant to maintain its natural defences. Possibilities include the use of non-host plants as intercrops, patches or borders to alter the volatile profile of the crop and act both as a repellent and an agent masking the host plant volatiles.

Within lavender essential oil, 29 main compounds were detected (Figure 5.2) and characterised by GC-MS. These compounds included a range of volatile monoterpenes, alcohols, esters, ketones, sesquiterpenes and oxygenated derivatives. Of these 29 compounds, the antennae of *M. aeneus* produced an EAG in the presence of 15 of them as shown by the GC-EAG recordings, which detected the change in receptor potential in the antenna.

Both the male and female antennae showed strong electrical responses to the overall lavender odour in the EAG studies, indicating that both sexes are capable of detecting non-host plant odour. Females were chosen for the behavioural bioassays as they are of most importance in pest control, and cause more damage to the oilseed rape plants during oviposition and feeding than the males cause during feeding. The possibility of sexual differences in *M. aeneus* responses to non-host plant odour is discussed in section 4.5.

The ability to detect chemicals via olfactory receptors does not necessarily imply a role of these cues in the ecology of the insect. Behavioural bioassays are required to establish the behavioural effects, if any, of each chemical. The results show that linalool, and its derivative linalyl acetate, were the two most repellent compounds of the 7 tested. These compounds occur in lavender in the highest concentration and this may explain the repellency to lavender, as even attractant odours can become repellent at high concentrations (Dethier, 1947). Both compounds are floral volatiles, found ubiquitously in the plant kingdom, and different concentrations result in attraction or repellence to a variety of insects. There are many references to their importance in plant-insect interactions. Indeed, linalool is found in the volatiles released by oilseed rape, and in field trials using baited water traps, linalool was shown to be an attractant to *M. aeneus* (Smart & Blight, 2000). However, the repellency shown in the research in this chapter may not be simply due to concentration effects. Smart and Blight (2000) showed that *M. aeneus* was attracted to linalool in the presence of oilseed rape volatiles, as the traps were placed on the edge of an oilseed rape field. Therefore, the presence of a large amount of isothiocyanates or other host plant volatiles might override the repellent effect.

There is the further possibility that specific ratios of linalool and linalyl acetate are responsible for the repellent effect of the lavender blend. It would be of interest in future studies to test the response of *M. aeneus* to varying ratios of these compounds to determine whether there is a point where maximum repellency is observed. An insect can determine ratios of volatile chemicals by comparing the stimulation of one type of receptor cell with that of another (Blight *et al.*, 1995c). For example, recordings from GC-SCR (single cell recording) (Wadhams, 1990) identified 166 responding olfactory cells in the antennae of *C. assimilis*, most of which showed high specificity in their response profiles (Blight *et al.*, 1995c). There were consistent pairings of specific cell types, such as methyl salicylate with 2-phenylethyl isothiocyanate, which indicates that specific ratios of these compounds are

important to the ecology of *C. assimilis*. Electrophysiological studies of the responses of *M. aeneus* to plant volatiles have not yet shown the existence of paired receptor cells on their antennae (Blight *et al.*, 1995b), however detailed SCR studies have not been attempted. Such GC-SCR studies could also help to establish whether the repellent non-host plant volatiles identified in this chapter are detected by *M. aeneus* using specialised olfactory cells, such as those identified in the ambrosia beetle, *Trypodendrum lineatum,* that responded exclusively to volatiles from birch, a non-host plant (Tommeras & Mustaparta, 1989).

Due to the large range of plants that release linalool, a broad range of effects is observed in many different insect species. For example, linalool is a component of alfalfa floral volatiles and was the only component within the blend that was shown to be attractive to honeybees (Henning *et al.*, 1992a). The green capsid bug, *Lygocoris pabulinus,* also uses linalool as a host-plant cue (Groot *et al.*, 1999). Repellency to linalool has been shown in cabbage butterfly (*Pieris rapae*) adults and gypsy moth (*Lymantria dispar*) larvae in response to the linalool component of non-host plants (Markovic *et al.*, 1996; Omura, 2000). Linalool is also a component of many insect pheromones, including male produced cabbage looper moth sex pheromone (Heath *et al.*, 1992) and a female attractant of the spined soldier bug (Aldrich *et al.*, 1984). Linalool has also been shown to reduce aphid catches when in combination with host- plant volatiles (Chapman *et al.*, 1981).

Other biological effects of linalool include antimicrobial activity. For example, linalool is released at an elevated rate by peanut plants on infection by white mould fungus, *Sclerotium rolfsii*, as a direct defence against pathogen attack and linalool was shown to inhibit fungal growth in culture (Cardoza *et al.*, 2002). This is a reminder that volatiles are often found in the essential oils of the vegetative parts of the plant and those involved in insect attraction or deterrence may actually serve multiple functions for the plant (Dobson, 1994).

Although anecdotal and despite the lack of statistically significant avoidance patterns by *M. aeneus* to 3-octanone in the 4-arm olfactometer tests, a clear behavioural pattern (turning away at the boundary of the odour stream) was observed. This is a general fungal volatile (Cardoza *et al.*, 2002). Detection of this volatile by *M. aeneus* could provide a general mechanism for avoidance of fungal infected host plants. 3-Octanone also occurs in

alfalfa floral volatiles and was repellent to honeybees when tested alone (Henning & Teuber, 1992b).

Thus far, it has proved difficult to find a chemical that acts as a repellent to all insect species when in competition with attractant volatiles. For example, methyl salicylate, a volatile derivative of salicylic acid, has been shown to act as a common insect repellent, and induces secondary metabolic defence based mechanisms in plants. Many aphid species have been shown to utilise this volatile as a non-host cue (Hardie *et al.*, 1994; Pettersson *et al.*, 1994; Glinwood & Pettersson, 2000a), and repellency to methyl salicylate is also seen in the biting midge, *Culicoides impunctatus* (Blackwell *et al.*, 1997). However, it is attractive to both *M. aeneus* and *Ceutorhynchus assimilis* (the cabbage seed weevil) (Smart *et al.*, 1995; Bartlet *et al.*, 1997).

It is important to understand insects' responses to a specific chemical in the context of co-occurring volatiles, in order to be able to predict the insects' behavioural response. An example where unexpected responses were found was when a bee repellent, 2-heptanone, was discovered in laboratory bioassays and then tested as a field spray to keep bees away from insecticide treated crops. The repellent effect in the field was very short lasting and even switched to attraction (Rieth *et al.*, 1986). Overall, the response of an insect to a chemical is very specific and shows considerable plasticity according to physiological conditions, concentration, season and life stage amongst many other factors. Therefore, it is important to consider many factors when investigating olfactory responses, and also to remember that extrapolation of responses between species and scenarios cannot be assumed.

As described in Section 1.4.7, there are several push-pull systems that have been field-tested along with the establishment of the chemical basis for their efficacy against the pests. For two of the systems, the 'push' comes from the use of neem-based antifeedants, extracted from the Neem tree, *Azadirachta indica* (Pyke *et al.*, 1987; Smart *et al.*, 1994). Although the neem extract reduced damage in field trials, no behavioural data is presented to prove that the assumed active ingredient, Azadirachtin, was responsible for this effect. Oviposition deterrents were used as the 'push' in the onion maggot control system and the most active materials proved to be derivatives of cinnamaldehyde (Miller & Cowles, 1990). The 'push' in a German cockroach system was methyl neodecanamide, that was

chosen from the literature as a general insect repellent (Nalyanya *et al.*, 2000). Khan et al (2000) adopted a different approach to finding a 'push' element to control stem borers pests of maize and sorghum. Hundreds of non-host plant species were grown and assessed for their effect on stem borer oviposition, and from these, several plants were selected for their low levels of attack. The volatiles from the selected non-host plants were analysed to identify compounds that did not occur within the host-plant volatile profile. These were then tested for behavioural activity against the pest. Therefore, the work in this thesis represents to date the most complete presentation of a rational approach to investigating the chemical basis of repellent non-host plant volatiles for use within a UK push-pull system. The use of this approach, as a model system for assessing the behavioural effects of repellent plant volatiles on insects and in the development of a push-pull system of control for *M. aeneus,* is discussed in Chapter 9.

The repellency of lavender essential oil as a model, non-host plant odour has been established in Chapters 3 & 4 (stage 1) and the detailed chemical basis established in this chapter (stages 3 & 4 in Poppy's 1991 sequence of experiments in semiochemical research). From this point on, lavender essential oil is used in semi-field (Chapter 6) and field experiments (Chapter 7) to complete Poppy's final stages of semiochemical research.

CHAPTER 6. SEMI-FIELD EVALUATION OF LAVENDER ESSENTIAL OIL AS A MODIFIER OF *MELIGETHES AENEUS* BEHAVIOUR

6.1 INTRODUCTION

Host-location behaviour in insects has been studied in the laboratory by exposing the insects to a range of host and non-host plant volatiles and observing their responses (Nottingham *et al.*, 1991; Bartlet *et al.*, 1993; Ruther & Thiemann, 1997). Laboratory bioassays have shown that *M. aeneus* is strongly repelled by the odour of lavender essential oil (chapters 3 & 4). However, in these bioassays the artificial environment of the laboratory may have affected the beetles' behaviour, but more importantly, the beetles were only assayed while walking and in the field *M. aeneus* are known to fly to their host plants (Williams & Free, 1978; Sedivy & Kocourek, 1994). The flight behaviour of a range of insects has been studied in the laboratory using specialised wind tunnels (Nottingham & Hardie, 1993; Glinwood *et al.*, 1999; Hurtrel & Thiery, 1999) and three dimensional flights patterns have been recorded using video techniques (Hardie & Young, 1997; El-Sayed *et al.*, 2000). However, the study of *M. aeneus* flight behaviour in a laboratory arena (free-flight in the air funnels – see chapter 3) was unsuccessful, as the beetles preferred to walk around the walls of the arena, despite having the space to fly (A. L. Mauchline, unpublished data). Evidently, the artificial environment of the laboratory is not conducive to flight in these beetles.

Due to the problems involved in studying the flight behaviour of *M. aeneus* in the laboratory and because the beetles only had a few cues in the laboratory compared to field conditions, their host-location behaviour during flight was studied in this chapter at a larger scale and under field conditions. Semi-field assessment is an integral part of pesticide testing for lethal and sub-lethal effects on insects (Kennedy *et al.*, 1996) and most laboratory experiments are first expanded to this intermediate step before moving up to the field scale (Hassan *et al.*, 1994; Sterk *et al.*, 1999; Umoru & Powell, 2002). The advantage of the semi-field scale is that the insects are free to perform their natural behaviour (except long distance flight) under field conditions, yet are usually confined within small arenas and therefore within view of the observer (Aluja & Prokopy, 1993). Due to the small size and slippery cuticle of *M. aeneus* it would be impractical to attempt a large-scale mark-

release experiment in the field. Therefore, the release of a known number into a confined space allows investigation of their movements and host-plant choices under field conditions (Hori, 1998). Insect-proof cages have been used to contain *M. aeneus* in order to study their susceptibility to entomopathogenic fungi (Butt *et al.*, 1998) and to assess yield loss caused by pollen beetle damage (Sylven & Svensson, 1976). In this chapter, the cages were used as large-scale arenas in which choice and no-choice tests using non-host plant odours can be performed under field conditions. Field conditions include the complex patterns of wind movement and speed that an insect is likely to encounter when trying to locate a host plant. Although the wind movements are not easily recorded, these cages allow observation of realistic field behaviour.

Techniques used for monitoring the flight behaviour of insects in the field are discussed further in Section 8.1, however many of these methods infer flight behaviour from the resultant distribution pattern rather than observation of movements of individuals. Field observation of individual insects is limited because of the difficulty in keeping track of dispersing individuals. Video (Riley *et al.*, 1990; Vickers & Baker, 1997) and radar techniques (Wallin & Ekbom, 1994; Osborne *et al.*, 1999) have been developed to follow individual insects, in particular butterflies and bees, which produces tracks of flight paths. More simplistic methods of direct observation over short ranges have been attempted for butterflies (Conradt *et al.*, 2000), but small insects can only be observed over very short distances.

Detailed observations of host-plant selection by the cabbage root fly have produced a clear description of the different stages involved for this species (Kostal & Finch, 1994). Borg and Ekbom (1996) have characterised the oviposition behaviour of *M. aeneus* by observing the behaviour of an individual within a cage containing host plant racemes. The individual was followed after moving onto the raceme and several components of the oviposition behaviour were distinguished and quantified. However, there has not been a detailed study of the flight movements and stages of host-plant location behaviour of *M. aeneus.* Therefore, in this chapter a method was developed to allow observation of flights of individual beetles. This led to the characterisation of host location behaviour and enabled identification of alterations in this behaviour in the presence of non-host plant odour.

Insects use a combination of olfactory and visual cues during host-location and acceptance behaviour. In order to establish when olfaction is of most importance deviations from the usual behavioural pattern, in response to introduced volatiles, need to be identified. There is a theory, put forward by Finch and Collier (2000), that during host-plant searching, insects land indiscriminately on green objects, such as leaves of host plants (appropriate landings) and non-host plants (inappropriate landings), but avoid landing on brown surfaces, such as soil. This mechanism suggests that only visual cues are important during the approach to a potential host plant and olfactory cues are only of importance after landing on a plant. This mechanism was used to explain the findings that the host location ability of a range of insects colonising vegetable crops were adversely affected when the host plants were surrounded by clover rather than bare soil (Kostal & Finch, 1994). Finch and Collier (2000) have put forward this behavioural model as a simplistic mechanism that is applicable to all species. However, as already detailed in chapter 5, responses to odours are very species specific and therefore the balance of visual and olfactory cues is also likely to vary enormously between species and situations. Therefore, it is of interest to establish the importance of olfactory cues for *M. aeneus,* both for the understanding of this behaviour, as well as to establish the most effective methods of using repellent odours in pest control.

6.2 AIMS

The first aim of the work in this chapter is to determine whether lavender essential oil, as a model non-host plant odour, has the same repellent effect observed in the laboratory, on *M. aeneus* while flying in field conditions. The host location behaviour of *M. aeneus* is then investigated further by splitting it into separate steps to establish the points at which olfaction is of most importance. This is achieved in two ways, firstly by altering the point at which the repellent cue is introduced, and secondly, by actually observing the differences in behaviour of the beetles in the presence of repellent odour.

1. To investigate the effect of non-host plant odours on host location and acceptance of *M. aeneus* under field conditions (Experiments 1 & 3).
2. To investigate the olfactory and visual mechanisms of host location and acceptance in *M. aeneus* by analysing their response to repellent odours at different points in the behavioural process (Experiment 2).
3. To characterise the host-plant location flight patterns of *M. aeneus* on approach to a host plant (Experiment 4).

6.3 MATERIALS AND METHODS

6.3.1 Experiment 1. Semi-field choice test to investigate differences in colonisation of lavender-treated and untreated oilseed rape plants

A metal-framed cage, dimensions 2.7 m x 2.7 m x 1.8 m high (Simpers Ltd., Cambridge, UK) was erected and a sealed, insect-proof mesh (Tygan™), cover suspended from the frame (Figure 6.1) (Appendix 2.). Two glasshouse-grown (Section 2.1), potted oilseed rape plants were placed on the ground in each corner of this arena (Figures 6.2 & 6.3). The plants were in full flower and the growth stages were balanced as far as possible to provide an equal stimulus in each position.

Figure 6.1 Semi-field cage

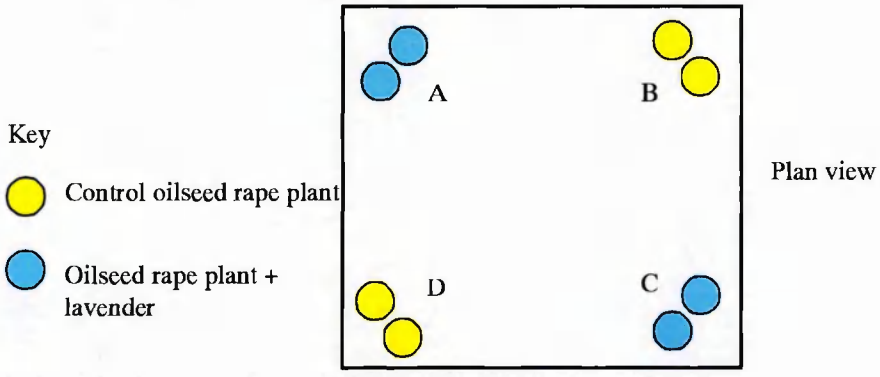


Figure 6.2 Layout of oilseed rape plants in the semi-field cage

Sachets were used to provide the treatment odour close to the potted plants. The lavender sachets were 250G polythene containing 0.3 ml pure lavender oil (Botanix) on sponge pieces (Section 2.4.1). The control sachets were 250G polythene containing untreated sponge pieces. For each potted oilseed rape plant, 5 sachets were attached to canes (three 1.5 m tall and two 1 m) pushed into the soil in each pot (Figure 6.3). This ensured that the sachets were hung at various heights near the flowers. The pots marked in blue in Figure 6.2 had lavender sachets suspended from each cane, and those marked in yellow had a control sachet suspended from each cane.

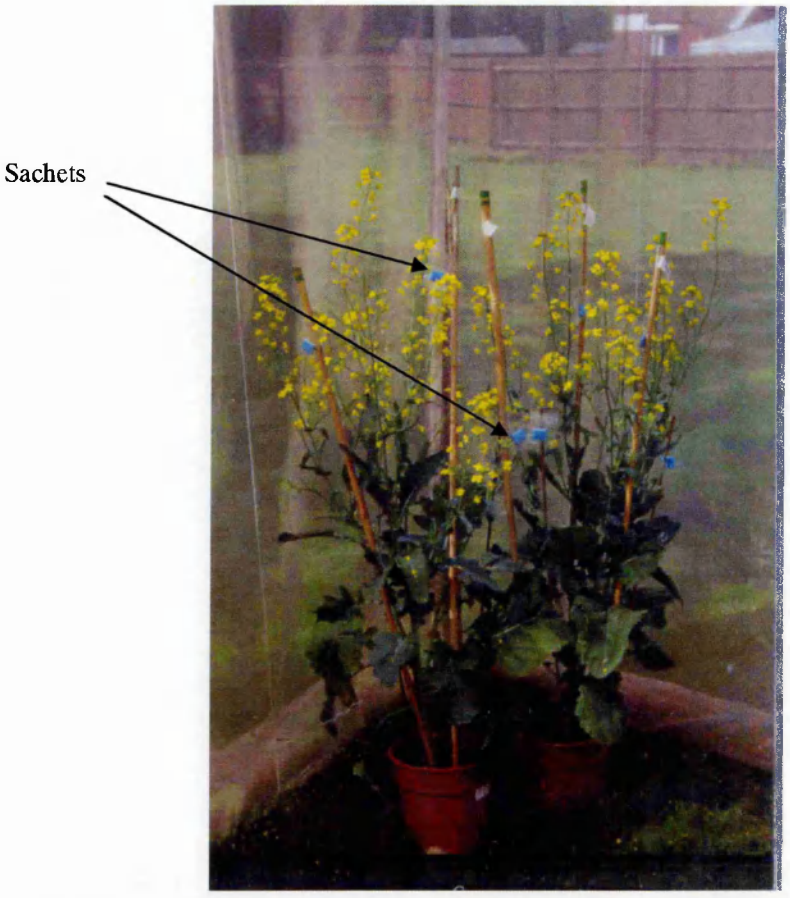


Figure 6.3 Potted oilseed rape plants in the semi-field cage, showing position of sachets

Meligethes aeneus were collected from flowering spring rape (Section 2.2.1). The beetles were left unsexed and starved overnight in empty plastic sandwich boxes (175 x 115 x 60 mm).

Five hundred *M. aeneus* were released from a sandwich box on the ground in the centre of the cage. A visual count of the number of beetles on each plant was taken 1, 3, and 6 hours after release. Twenty four hours after release, the beetles were collected from the plants using an electric pooter and counted.

Ten replicates were conducted and between replicates all the beetles from within the cage were removed and the position of the treatments was alternated.

6.3.2 Experiment 2. Investigation into the timing of the action of lavender odour during host location by *M. aeneus*

The protocol for experiment 1 was repeated with the following amendments. The plants and equipment were positioned in the cage (Figure 6.1) and five canes were placed in position in the pots of oilseed rape, but without the sachets.

Five hundred *M. aeneus* were released from a sandwich box on the ground in the centre of the cage. A visual count of the number of beetles on each plant was taken after an hour. At this point the sachets were suspended from the canes. The sachets were prepared in the same way as experiment 1 (section 6.3.1). After a further hour (2 hours after insect release), a visual count of the number of beetles on each plant was made and beetles were collected from the plants using an electric pooter. Ten replicates were conducted.

6.3.3 Experiment 3. Semi-field no-choice test to investigate colonisation patterns of lavender-treated and untreated oilseed rape plants

The protocol for experiment 1 was repeated but two cages were set up as shown in Figure 6.1. One cage contained control plants in all positions and the other cage contained plants with lavender sachets in all positions. This was designed as a no-choice test. Five hundred *M. aeneus* were released from a sandwich box on the ground in the centre of the cage. A visual count of the number of beetles on each plant was taken 0.25, 0.5, 1, 2, 4, 6 and 24 hours after release. Eight replicates were conducted.

6.3.4 Experiment 4. Observations of *M. aeneus* flights towards lavender-treated and untreated oilseed rape plants

Observations of the host-location behaviour of *M. aeneus* were conducted in the metal-framed, mesh-covered cage (Figure 6.1). The layout of the plants was different to the previous experiments; only two flowering, potted oilseed rape plants (Section 2.1) were used and were both placed on the ground next to each other in *one* corner of the cage. Five lavender essential oil sachets were hung on one plant and 5 control sachets were hung on the other, giving an even coverage over the plants. The sachets were made using 0.3 ml lavender oil on a sponge contained in 250G polythene (Section 2.4.1).

Meligethes aeneus adults were collected from spring rape (Section 2.2.1). The beetles were left unsexed, and starved overnight in empty sandwich boxes.

Two hundred beetles were released from a box placed on the ground in front of the potted plants, between the observer and the plants. The flights of the beetles were observed for six periods of one hour each and descriptions of all flights seen were recorded on a dictaphone. The dictaphone records were transcribed and the following parameters recorded for each flight:

- time of flight within the hour
- landed on a plant? (yes/no)
- landed on which plant (control or lavender-treated plant)
- where landed? (flower, bud, leaf, stem, other)
- direct or hovering/lowering flight?
- time spent hovering
- route of flight in relation to the control and treated plants
- whether it flew close to a different plant before landing

Also, the final number of beetles on both plants were counted at the end of each observation.

6.3.5 Statistical analysis

6.3.5.1 Experiment 1. Semi-field choice test to investigate differences in colonisation of lavender-treated and untreated oilseed rape plants

The difference in the number of beetles on the lavender treated and control plants was analysed using a paired t-test. Multiple regression analysis was used to investigate the influence of total beetles settling and meteorological factors (wind speed and maximum daily temperature measured at the Rothamsted meteorological station ~ 200 m away) on the proportion of colonising beetles landing on the lavender-treated plants.

6.3.5.2 Experiment 2. Investigation into the timing of the action of lavender odour during host location by M. aeneus

A paired t-test was used to analyse any treatment differences in the number of beetles on the control and lavender treated plants one hour after application of the treatments.

6.3.5.3 Experiment 3. Semi-field no-choice test to investigate colonisation patterns of lavender-treated and untreated oilseed rape plants

Differences in the number of beetles on the control and lavender-treated plants over time were analysed using repeated measures analysis of variance. This form of repeated

measures data can be regarded as a split-plot experiment with beetle counts as whole plots and days as subplots. Tests were done to assess correlation between adjacent time periods and the degrees of freedom in the subplot stratum of the split-plot analysis were adjusted by the correlation factor epsilon before treatment differences were tested.

6.3.5.4 Experiment 4. Observations of M. aeneus *flights towards lavender-treated and untreated oilseed rape plants*

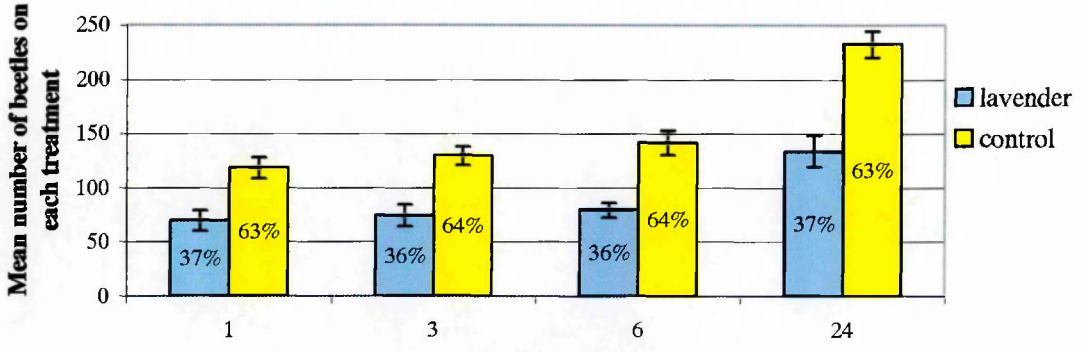
Due to the different number of replicates for each behaviour, the data for the six hours of observation were summed and separated into different categories. The data are thus presented as a description of the behaviour. Chi-square analysis was conducted to establish whether there was any difference between the total number of insects landing on the control and treated plants after hovering (control=72, treated=56) or direct (control=108, treated=52) flights.

6.4 RESULTS

6.4.1 Experiment 1. Semi-field choice test to investigate differences in colonisation of lavender-treated and untreated oilseed rape plants

The mean number of beetles on the lavender-treated plants was consistently lower than on the control plants throughout the experiment (Figure 6.4). Twenty four hours after release the number of beetles on the lavender-treated plants (133.7 ±14.6) was significantly different to that on the control plants (232.6 ±11.9), (t-test p=0.0013 t=-4.58 d.f.=9). The ratio of beetles on the lavender-treated plant compared to the control plant remained very constant throughout the 24 hour period of the experiment at about 36%:64% respectively (Figure 6.4). This is mainly due to the fact that the majority of beetles that landed in the first 6 hours, landed within the first hour after release and the ratio on the plants was therefore maintained. The apparent increase in the number of beetles on the plants after 24 hours was due to the increased efficiency of the sampling method (electric pooter compared to previous visual counts), but the ratio was conserved indicating that it was a true trend throughout the sampling period. This method for analysing the host location behaviour of *M. aeneus* was very suitable as an average recapture of 73% was achieved 24 hours after release.

Figure 6.4 Settling pattern of *M. aeneus* in semi-field experiment 1 (±SE) (n=10)



Numbers on bars represent percentages of all beetles on plants

There was interesting variation in the data. There were 6 replicates where the lavender:control ratio was approximately 30:70 but there were 4 replicates when the ratio was approximately 45:55 (Table 6.1). Regression analysis showed that the reduced ratios in these 4 replicates was not due to overcrowding on the control plants, forcing movement onto the lavender treated plants (p=0.320). However, a meteorological factor, wind speed, did show a significant influence on the settling pattern; at higher wind speeds, a higher proportion of colonising beetles landed on the lavender-treated plants (p=0.031). Maximum daily temperature, was negatively correlated with proportion of colonisation on the lavender-treated plants, but this was not significant (p=0.061).

Table 6.1 Dates of replicates of experiment 1 with maximum daily temperatures and mean wind speeds

Replicate (24 hours)	Date	Maximum daily temperature (°C)	Mean daily wind speed (m/s)	Ratio of insects on treated plants: untreated plants
1	26 June 2001	27.4	2.37	36:64
2	27 June 2001	23.4	2.57	48:52
3	28 June 2001	22.6	2.21	44:56
4	3 July 2001	26.4	2.05	30:70
5	4 July 2001	27.4	2.29	32:68
6	5 July 2001	28.9	2.14	30:70
7	6 July 2001	26.0	0.97	22:78
8	11 July 2001	17.6	4.40	44:56
9	12 July 2001	19.5	2.98	47:53
10	17 July 2001	20.1	2.69	27:73

6.4.2 Experiment 2. Investigation into the timing of the action of lavender odour during host location by *M. aeneus*

Before the application of the treatments, the mean numbers of beetles on the two sets of plants were similar (set 1 – later treated with lavender 82.2 ±4.6 and set 2 – later treated as the control 75.2 ±3.0). One hour after application of the treatment sachets, the means were still very similar (lavender=89.3 ±5.5, control=80.2 ±5.6). A paired t-test showed no significant difference between the means on the two treatments one hour after the treatment was applied (p=0.128, t=1.68, d.f.=9). These data are shown in Figure 6.5 as proportions of the total number of colonising beetles. As for experiment 1, the majority of beetles that colonised the plants during the experiment, landed within the first hour after release. Few landed on either treatment during the second hour of the experiment. All these replicates were conducted under similar meteorological conditions (Table 6.2).

Figure 6.5 Mean number of beetles (±SE) before and after treatment in semi-field Experiment 2 (N=10)

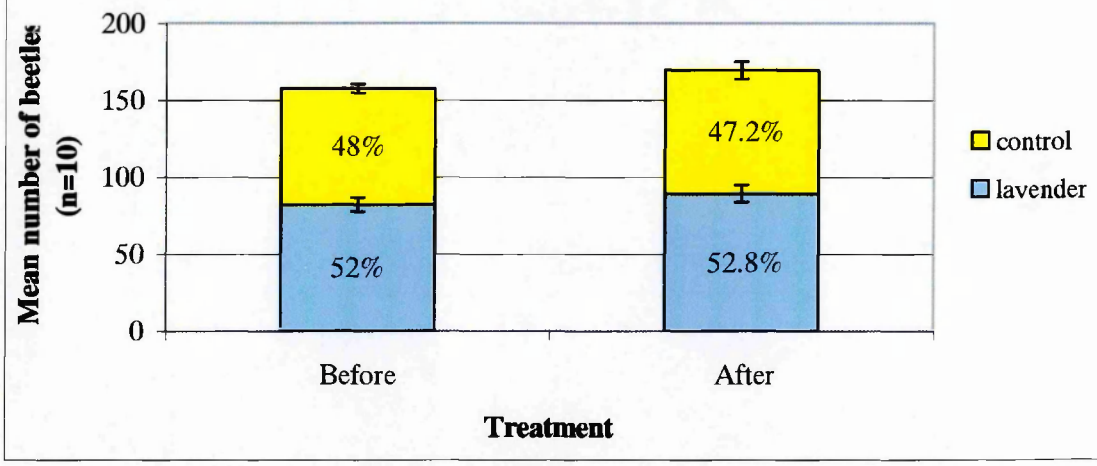


Table 6.2 Dates of replicates of experiment 2 with maximum daily temperatures and mean wind speeds

Replicate (2 hours)	Date	Maximum daily temperature (°C)	Mean daily wind speed (m/s)
1	25 July 2001	25.0	1.25
2	25 July 2001	25.0	1.25
3	26 July 2001	27.1	0.95
4	26 July 2001	27.1	0.95
5	27 July 2001	27.6	0.78
6	27 July 2001	27.6	0.78
7	27 July 2001	27.6	0.78
8	28 July 2001	29.8	0.69
9	28 July 2001	29.8	0.69
10	28 July 2001	29.8	0.69

6.4.3 Experiment 3. Semi-field no-choice test to investigate colonisation patterns of lavender-treated and untreated oilseed rape plants

In this no-choice experiment, the number of beetles on the lavender-treated plants was significantly lower than on the control plants over the 24-hour experimental period (Figure 6.6) ($F_{1,14}$=17.29 p<0.001). The numbers on both treatments rose significantly over the 24 hour period ($F_{2.9,40}$=36.45 p<0.001) however the difference between the treatments did not vary over time ($F_{2.9,40}$=0.11 p=0.95).

Even after 24 hours, there was still a large proportion of the beetles in the lavender-treated cage that were not on the plants, despite a lack of alternative host plants. On average, 35% of the 500 beetles in the control cage had colonised the plants after 24 hours, compared to only 25% in the lavender cage – this is a difference of approximately 50 beetles, which is almost a third of all beetles that colonised the plants in the control cage. All replicates were conducted under similar meteorological conditions (Table 6.3).

Figure 6.6 Mean number of beetles on each treament in semi-field experiment 3 (N=8)

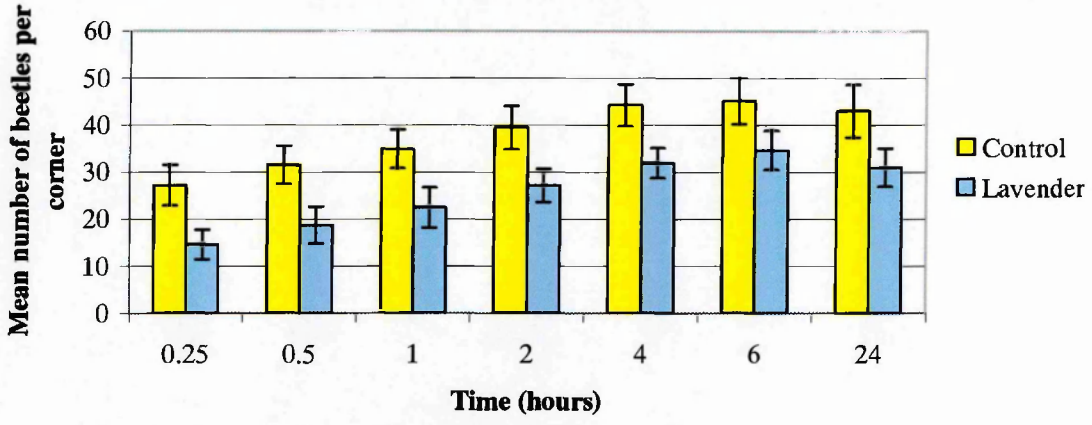


Table 6.3 Dates of replicates of experiment 3 with maximum daily temperatures and mean wind speeds

Replicate (24 hours)	Date	Maximum daily temperature (°C)	Mean daily wind speed (m/s)
1	31 May 2002	18.3	1.30
2	1 June 2002	20.8	1.90
3	2 June 2002	25.1	1.84
4	18 June 2002	21.2	1.67
5	19 June 2002	20.3	1.06
6	16 July 2002	23.6	1.31
7	17 July 2002	20.5	1.56
8	30 July 2002	25.7	0.78

6.4.4 Experiment 4. Observations of *M. aeneus* flights towards lavender-treated and untreated oilseed rape plants

Flights were observed as the insects approached the plants. Due to their small size, the beetles were camouflaged by the surroundings until they were within approximately 1 metre of the observer. Therefore, all flights that could be seen were recorded, but some are likely to have been missed due to more than one insect flying at any given time point. Only 47 out of 288 landing beetles were seen taking off again, but these secondary flights are not presented here as numbers were low and the experiment was only examining the beetles' initial response to the plants. Table 6.4 shows the date and meteorological conditions for the observations.

Table 6.4 Dates of replicates of experiment 4 with maximum daily temperatures and mean wind speeds

Replicate (1 hour)	Date	Maximum daily temperature (°C)	Mean daily wind speed (m/s)
1	16 July 2001	20.5	0.89
2	17 July 2001	20.1	2.69
3	20 July 2001	19.9	1.97
4	25 July 2001	25.0	1.25
5	26 July 2002	27.5	1.45
6	1 August 2002	19.9	0.59

The data from these observations are described as the total number of flights in each category as shown in Figure 6.7. Three hundred and sixty-four flights were observed during the six hours and are described to provide preliminary data to back up the results from experiments 1, 2 & 3 in this chapter. An average of 60 flights were recorded within the one-hour observation. Of all those flights, 288 (79%) resulted in the beetle landing on a plant and 76 (21%) continued flying without being observed to land. Of those 288 that landed, 180 (63%) landed on the control plant whereas only 108 (37%) landed on the lavender-treated plant. The majority of the landings (79%) were on the flowers (as opposed to any other part of the plant) regardless of treatment.

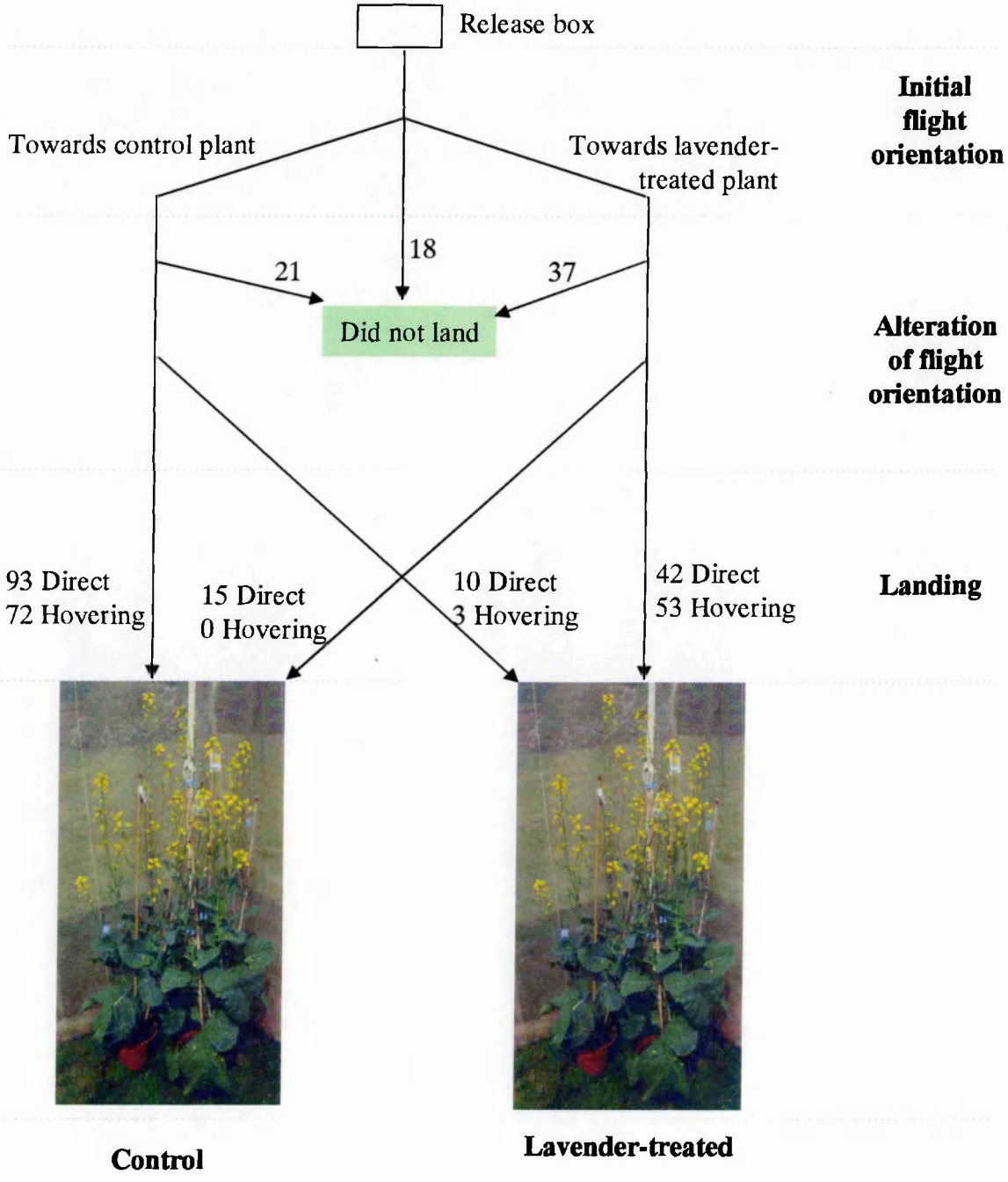
Hovering (flight without vertical or horizontal movement) and lowering flight (zig-zag flights towards the ground) behaviour was recorded to investigate how free-flying beetles respond to the plant at close range. Almost half of all flights that resulted in a landing (44%) were after more than 3 seconds of hovering near the plant. Fifty two percent of flights that landed on the lavender-treated plant involved hovering, compared to only 40%

of flights that landed on the control plant (df=1, χ^2=3.84, p=0.05) (see summary in box at bottom of Figure 6.7).

Seventy six (21%) of the flights did not result in an observed landing. These flights included flying straight past or hovering near a plant without landing. The flight path of the majority of these non-landing flights (48%) were in the vicinity of the lavender treated plant, whereas only 28% were near the control plant and 24% flew past both. This high proportion of non-landing flights occurring near the lavender-treated plant may indicate that these insects are able to alter their flight course according to the olfactory cues gained on approach to the plant.

In summary, more flights were observed to result in a landing on a control plant (63%) compared to a lavender-treated plant (37%). This was partly accounted for by beetles flying towards a lavender-treated plant but not landing. The cues detected during the approach flight are likely to have a strong olfactory element (although it is unclear how far the non-host odour emanated from the sachets) as the visual cues were standardised as far as possible. The wind direction within the cages was not measured, but the plants were located immediately next to each other and therefore the wind conditions were likely to be similar for both treatments. Hovering was investigated as a possible behaviour that would enable the insects to assess the olfactory stimulus from each plant. The highest proportion of hovering, non-landing flights occurred in the vicinity of the lavender-treated plant.

Figure 6.7 Representation of observed flight movements
The number of flights in each category are shown. The landing flights are classified as either direct (D) or hovering (H).



Landing flights	Control	Lavender-treated
Direct	108	52
Hovering	72	56

6.5 DISCUSSION

The choice test (experiment 1) was designed to determine whether *M. aeneus* could detect lavender odour released from sachets under field conditions and whether this detection led to a decrease in host-plant colonisation rate in comparison with an untreated control (visual cues between treatment and control were kept as consistent as possible). The data suggest that the beetles could detect the lavender odour and preferentially colonised the untreated control plants. This provides additional evidence that lavender is repellent to these beetles during host location/acceptance behaviour. This also indicates that olfactory cues are of importance in this behavioural process and that repellents are able to have an effect while the beetles are flying, as well as walking (Chapter 4), towards their potential host plants. The beetles are known to fly to their host plants in the field as they have been observed to fly between plants within oilseed rape fields (personal observation) and are not caught in pitfall traps (D. Warner, personal communication).

Experiment 2 was designed to examine the steps involved in host location/acceptance by *M. aeneus* and establish the point at which lavender has a repellent effect. Experiment 1 established that lavender odour, under field conditions, reduces the number of insects. By repeating this protocol, but delaying the treatment application until after host colonisation by a significant number of beetles, it was possible to show that lavender is not effective as a repellent after colonisation. It is likely that the beetles can still detect the lavender while on the plant, but the additional information gained from inspection of the plant is sufficient to identify it as the correct host, despite the presence of lavender odour. For example, taste (especially of sugars) is more important in the arrestment of pollen beetles on a plant than visual or olfactory cues (Charpentier & Charpentier, 1986). These findings are similar to a study on the aphid *Aphis fabae* where the repellent non-host volatile 1-heptanonitrile induced more avoidance flights and delayed stylet penetration, but not any subsequent behaviours. The authors suggested that after stylet penetration, sensory modalities other than olfaction become more important (Storer *et al.*, 1996). Whichever mechanism is occurring after arrival at a host plant, it is evident that olfactory cues are of importance during flight towards the host plant. This contradicts the theory of 'appropriate/inappropriate landings' which states that visual cues are the central link in the host-location behaviour of all insects (Finch & Collier, 2000). The central link in the behaviour of this species has been shown to be governed, at least in part, by olfactory

stimuli, however there is agreement that the final link is governed by non-volatile plant chemicals.

The no-choice test (experiment 3) aimed to show whether the beetles would over-ride their avoidance of lavender odour in the absence of other host plants and the results from this experiment have shown that this did not happen within the time period of the experiment. Even after 24 hours in the cage with the treated plants, a large proportion of beetles preferred not to feed or oviposit on the treated plants and were found on the roof or sides of the cage at the end of the experiment.

The step-wise sequence of behavioural events involved in host location by phytophagous insects ultimately leads to either feeding or oviposition behaviour. In most insects, a delay in achieving one of these behaviours due to a lack of suitable host plants changes the physiological state of the insect (Courtney *et al.*, 1989). However, studies by Hopkins (1996; 1999) have shown that low host quality or low host encounter rates lead to a reduction in egg production and accumulation by *M. aeneus*. This means that *M. aeneus* does not show an increased requirement for oviposition over time. These results could explain why there was no increase in beetles settling on the treated plants over the 24-hour period. Those females that are in the process of host location for oviposition sites, are under no time pressure to locate hosts as the reduction in egg production enables them to switch back to host-searching mode in the absence of good quality host plants. *Meligethes aeneus* is less selective over *Brassica* food host plants than oviposition hosts (Ekbom & Borg, 1996), therefore those insects that colonised the plants in the lavender-treated cage could be feeding rather than ovipositing. Future studies could investigate this by collecting the insects from the plants and the walls of the cage at the end of each replicate and sexing and dissecting the females to identify any differences in the spatial distribution of males, females and gravid females.

This finding is of importance in terms of assessing the use of repellent signals within the push-pull system. During the early stages of colonisation it is important to prevent the insects from establishing a population within the main area of the crop. The aim of repellents is to re-direct early arrivals into areas where they can be controlled. The results from experiment 1 have shown that lavender reduced the number of colonising insects by a third, indicating that during the 24-hour period some insects have been unsuccessful in

finding a suitable host plant. The most effective way in which repellent odours can reduce pest numbers in the field is to cause individuals to switch back to searching flights from host-finding behaviour, as this is likely to avoid the problem of inducing short flights which re-direct the pests into other areas of the crop (R. Potting, personal communication).

The behavioural observations in experiment 4 were conducted in order to investigate the avoidance behaviour in response to lavender odour. There were fewer beetles landing on the lavender-treated plants. Also, of those that landed on the lavender-treated plant a higher proportion of them hovered for an extended period before landing, in comparison to those landing on the control plants ($p=0.05$). It proved difficult to watch such tiny insects under semi-field conditions: future observations would need more accurate measurement of microclimatic conditions around the plant to properly assess the effect of wind and temperature on the behaviour.

As expected, the flowers of the oilseed rape plant were the most frequent landing sites in both treatments and this again shows the strong attraction, both visual and olfactory, of the flowers to these beetles (Charpentier, 1985). Because many beetles were alighting on the plants during the observations, it was impossible to identify individuals and assess the length of time spent on the plant after arrival. Further, more detailed, observations of the behaviour of individuals after landing would be required to understand the host acceptance behaviour for feeding and oviposition (Borg & Ekbom, 1996) in the presence of non-host plant odour.

This chapter is the first attempt to characterise the host location behaviour of *M. aeneus* under field conditions in an intermediate stage between steps 5 and 6 of Poppy's scheme (1991). The host location behaviour and immigration of populations of *M. aeneus* to fields of oilseed rape has been studied indirectly (Section 8.1.1), and this is studied in more detail in Chapter 8. The alteration of their response in the presence of non-host plant odour has also been shown. The application of non-host plant odour in the field is investigated in Chapter 7 in order to determine whether it is effective in affecting numbers of *M. aeneus* in fields of oilseed rape and whether this can actually alter crop yields.

CHAPTER 7. EFFECTS OF NON-HOST PLANT ODOUR TO *MELIGETHES AENEUS* DURING IMMIGRATION TO SPRING RAPE FIELDS

7.1 INTRODUCTION

Evidence from previous experimental work detailed in this thesis has shown that lavender essential oil reduces numbers of adult *M. aeneus* in confined experiments, both in the laboratory (Chapters 3 & 4) and semi-field cages (Chapter 6). An open field trial was required to establish whether this effect is apparent during the different phases of natural colonisation of an oilseed rape field by these insects. The flight patterns of *M. aeneus* at a range of altitudes are described in chapter 8, whilst in this chapter field experiments to test the effectiveness of lavender odour during crop colonisation are reported.

Two field methodologies are examined; use of baited traps and treatment of crop plants, both of which have been used by others to test the effectiveness of semiochemicals in the field (Smart *et al.*, 1997a; Smart *et al.*, 1994). The host preferences of phytophagous insects can be investigated using traps to catch flying insects in the vicinity of the crop. Water traps or sticky traps can be manipulated experimentally to present a variety of olfactory and visual cues. (Smart *et al.*, 1997a; Blight & Smart, 1999; Smart & Blight, 2000). The use of such techniques enables comparison of insect responses to a precise range of stimuli, but these responses of the insects are towards artificial targets and not to their host plants. Olfactory responses to volatile chemicals or plant extracts can be tested in this way, although responses to naturally released volatiles from whole plants can not be assessed.

Responses to whole plants such as field crops or stands of potted plants can be manipulated experimentally and the number of insects arriving at the different areas can be assessed (Ekbom & Borg, 1996). Intercropping (Hooks & Johnson, 2001) and trap cropping (Hokkanen *et al.*, 1986) experiments are examples where host preferences need to be assessed using whole plants. This avoids the problem of using artificial targets as the insects can orientate and land on the plants themselves. Also, *M. aeneus* is generally more attracted to its natural host plants than to any artificial device (Hokkanen *et al.*, 1986). A visual count of the numbers of adult insects on plants is a standard method to estimate the

population densities within different treatments (Williams & Free, 1978; Ekbom & Borg, 1996). Natural levels of immigration can be augmented by the release of either laboratory-reared or field-collected individuals into the experimental plots (Coll & Bottrell, 1996). More specifically, oviposition preferences of field populations can be investigated by dissecting field plants and counting the number of larvae (Khan *et al.*, 2000) and eggs (Uvah & Coaker, 1984).

It is important to anticipate the effects of semiochemicals when applied on a large field scale and to determine the optimal spatial deployment of trap crops and odours. A computer model has been developed to address such issues, which is based on individual insect movements in response to behavioural stimuli (Potting *et al.*, 2002). This simulation of the temporal and spatial dynamics of insect pests in oilseed rape will help in the implementation of the different elements of the push-pull strategy at the most effective point during the colonisation phase. Initial results of the simulation model show that a trap crop strategy is likely to be effective for controlling pests with good powers of dispersal and host-plant location, such as *M. aeneus*. The model also predicts that repellent odours can significantly reduce damage levels to the crop and would be most successful when they induce airborne emigration, rather than short-range, local movements.

Such predictions are difficult to evaluate in the field. Therefore, two, potentially useful methodologies were developed in the work described in this chapter to determine the effect of non-host plant odours on *M. aeneus* during host colonisation in the field. Both methods measure the preferences of flying insects to different odour treatments. The first method used yellow water traps with control, attractant (2-phenylethyl isothiocyanate) or non-host plant odours (lavender essential oil), which were placed in a field of spring rape. Secondly, a different field of spring rape was treated with control or non-host plant odour sachets in a replicated plot design. The effects of the treatments in each experiment were determined by counting insects in the water traps and assessments of the numbers of adults in treated and untreated areas of the field respectively.

7.2 AIMS

1. To evaluate two methods of field trials for investigating the efficacy of lavender odour for disrupting colonisation by insect pests in oilseed rape.
2. To establish whether non-host plant odour can be used to reduce immigration of *M. aeneus* into treated plots within fields of oilseed rape.

7.3 MATERIALS AND METHODS

7.3.1 Water trap experiment

Plastic water bowls (21 cm diameter, 9 cm deep) painted canary yellow (Smart *et al.*, 1995; Smart *et al.*, 1997a) were supported on the top of 1 m canes (Figure 2.4). Arrays of 16 traps (2 m apart) were set up in two fields on Rothamsted farm (Appendix 2.);
Claycroft - set up on 13th June 2001, completed 29th June 2001 (16 days).
Furzefield - set up on 14th June 2001, completed 29th June 2001 (15 days).

Both fields were sown with spring oilseed rape, but the crops were at early growth stages; pre-green bud i.e. no flower head development. Towards the end of the trapping period, some of the crop in Claycroft was beginning to flower, but the crop in Furzefield was still pre-green bud. *Meligethes aeneus* adults from both the old and new generations were present in the crop.

The bowls were filled with a mixture of water and detergent (Teepol) and emptied every few days over a 12-day period (Tables 7.1 & 7.2). When emptying the traps, the water was strained through a piece of muslin, retaining the insects. The insects were stored in alcohol in glass vials and the bowls refilled. The treatments were applied as sachets (section 2.5) fixed to plastic supports, which held the sachet just above the rim of the bowl (Figure 2.5). The four treatments were:

A Blank (empty sachet)
B Lavender low release rate (0.3 ml, 3 mm sponge, 2000 G) ~ 1 mg/day
C Lavender high release rate (0.3 ml, 3 mm sponge, 250 G) ~ 9 mg/day
D 2-phenylethyl isothiocyanate (0.5 ml, 3 mm sponge, 250 G) ~ 5 mg/day (attractant (Blight & Smart, 1999))

The treated traps were laid out in a Latin square design, for example:

D	C	A	B
B	A	C	D
C	D	B	A
A	B	D	C

The numbers of *M. aeneus* in the samples were counted using a dissecting microscope. The data were summed over the course of each experiment and the numbers of beetles caught per trap were analysed using a hierarchical analysis of variance with the treatment degrees of freedom partitioned into specific independent contrasts. Firstly, the number of beetles in the isothiocyanate traps was compared to the other treatments. Secondly, the blank control trap counts were compared to the two lavender treatments combined and then to the high concentration of lavender alone. Finally, the numbers of beetles in the two lavender treatments were analysed for concentration differences.

7.3.2 Field plot experiment

7.3.2.1 Treatments

A field of spring oilseed rape (variety Heros) was sown at 120 seed/m^2 on 22nd April 2002 in Great Harpenden I, Rothamsted farm (Appendix 2).

A continuous area of 29 m x 29 m was selected and within this area sixteen 1 m^2 plots were marked out, with 5 m between each plot (Figure 7.1). On the 19th June, when the crop was in early green bud, the plots were measured using a tape measure and marked out using plastic markers pushed into the soil. The design of the experiment was 8 blocks of two treatments (control or lavender treated) which were allocated on a checkerboard layout.

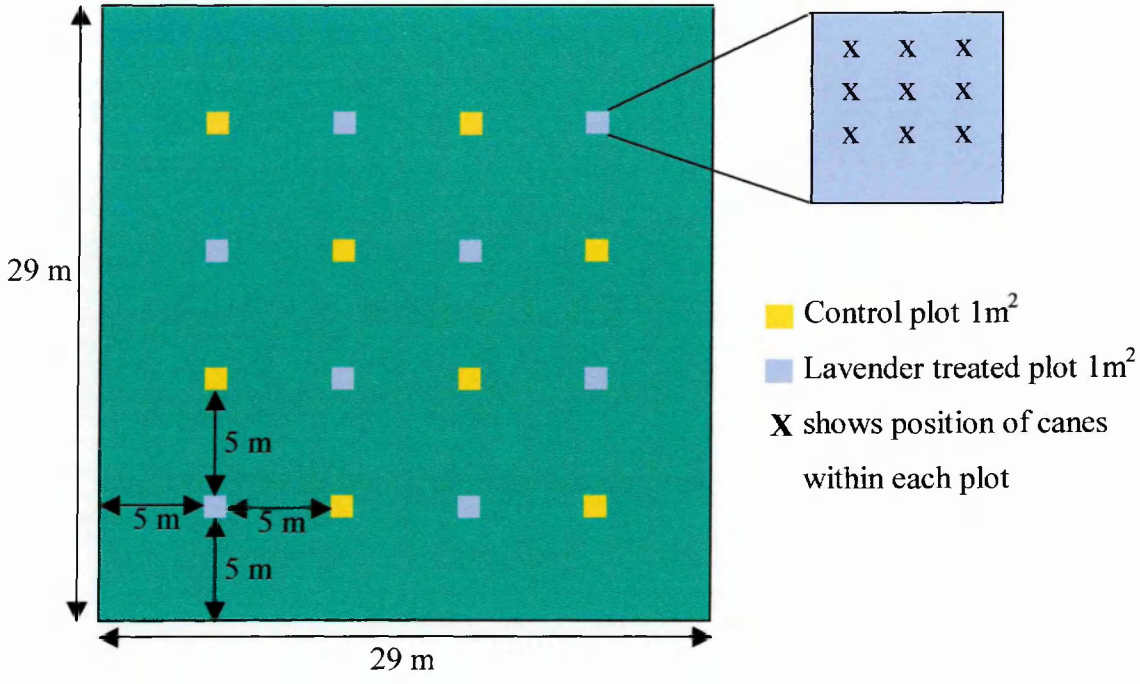


Figure 7.1 Field plan showing control and lavender treated plots (plan view)

Control and lavender sachets were made as described in section 2.5. Sachets were made using thin sponges with 250 G bags. 0.3 ml of Botanix lavender oil was added to each sponge. Within each plot, nine equally spaced 3-foot canes were pushed into the soil. A sachet was attached to each cane using gardening twist-ties at the same height as the developing flower heads of the crop (Figure 7.2). The lavender sachets were replaced weekly and the height adjusted to retain them at the height of the flowers.

7.3.2.2 Assessments of pollen beetle incidence and crop growth stage

Due to a delay at the start of the experiment, the crop became infested with low numbers of pests before the application of the treatments. Therefore, immediately prior to the application of treatments, all insects were removed from the experimental plots using an electric pooter. The 5m borders were not cleared and therefore provided a source of insects for recolonisation.

Figure 7.2 Field plot experiment showing oilseed rape plants in early yellow bud and position of treatment sachets on canes in one plot (1 m^2)

Assessments were made twice weekly. Ten plants per plot were assessed in the field (non-destructive sampling) and these were selected using randomly-generated x,y co-ordinates to prevent bias. The assessments consisted of counts of adult *M. aeneus* present on each plant and records of the overall growth stage of the oilseed rape plants using the code in Lancashire (1991) (see Table 2.3).

The growth stage (GS) assessment was amended on the 10th July. Instead of recording the overall growth stage of the plant as before, the growth stage of the 1st and 3rd racemes were recorded from this date onwards. This amendment was required to clarify emerging differences between plants, as some were beginning to compensate for severe insect damage to the main racemes, and this was complicating the overall growth stage assessment.

7.3.2.3 Bud samples

On the 1st July 2002, a bud sample was taken from each plot. The majority of the crop was at the yellow-bud growth stage. The main racemes from 5 plants in each plot were cut just above the point of branching into the secondary raceme. The samples were transferred to the laboratory where they were stored in pots of water and kept at 5°C. Using fine forceps,

each bud was removed from the stem and observed under a dissection microscope. The bud was examined and any insect damage was recorded. Each damaged bud was then dissected using two pairs of fine forceps and *M. aeneus* eggs and larvae were counted.

7.3.2.4 Yield analysis

The oilseed rape plants were harvested from the field on the 2nd September 2002 as the plants were beginning to turn brown. In order to do this, the crop was flattened along one edge of each plot. A 3-sided ½ m^2 quadrat (0.7 m x 0.7 m = 0.49 m^2) was then pushed into the plot at the base of the plant stems and used to mark out the area for harvest. All the oilseed rape plants in the area of the quadrat were then cut at the base of the stem. The plants were then placed in tall, clear plastic bags and stored at 5°C until examination.

The whole plot sample was weighed and the fresh weight recorded. Ten plants per plot were removed and placed in hessian sacks and returned to 5° C. The remainder of the sample was then chopped into small pieces and a sample placed in a metal tin and weighed. The sample was then dried in an oven at 80° C overnight. Dry weights of the sample were taken the following morning and the percentage dry matter calculated.

The 10 plants from each plot (stored in hessian sacks) were scored for plant architecture. The number of racemes bearing pods (or remnants of pods) and the total number of pods per plant were counted. The mean number of pods per raceme was then calculated.

After the architecture analysis, the pods from the main and 3rd racemes of 2 randomly selected plants per plot were removed and the number of seeds per pod counted. The 8 remaining plants were then returned to the hessian sacks and left at room temperature for 3 weeks to dry. The racemes were cut from all the plants and placed in a threshing machine, which was used to break open the pods. The seeds were separated from the plant debris using a gentle vertical airflow. One thousand seeds were counted and were weighed. The total weight of seeds from 8 plants per plot was also taken, and using the weight of 1000 seeds, the mean number of seeds per plant for each plot was estimated using the formula ((total weight of seeds/weight of 1000 seeds) x 1000)/8.

7.3.2.5 Statistical analysis

For all the parameters detailed above, the means per plot were calculated. For each assessment of adult beetle numbers on each date, the parameters taken for the bud samples and yield analysis per plot were analysed for treatment differences using analysis of variance in Genstat. The data was checked to ensure normality and equality of variance by plotting a histogram of the residuals and plotting the residuals against the fitted values respectively, using Genstat.

The growth stages of the oilseed rape plants were recorded using a scale with defined numerical codes, which were not on a continuous scale. Therefore the modal growth stage per treatment (n=80) was calculated and plotted to show the trends over the experiment. The non-parametric Mann Whitney test was used to analyse treatment differences in the oilseed rape growth stages, using all 80 plants for each treatment, on each date.

7.4 RESULTS

7.4.1 Water trap experiment

The mean number of *M. aeneus* in each treatment per sampling date and the mean total over the whole sampling period were calculated for both fields (Tables 7.1 and 7.2).

Table 7.1 Claycroft – Mean number of *M. aeneus* per water trap (±SE)

	Blank (Control)	Low lavender	High lavender	2-phenyethyl isothiocyanate
18/6/01	7 (± 1.1)	6 (± 1.5)	6.3 (± 1.2)	21 (± 2.9)
19/6/01	18.8 (± 3.9)	25.3 (± 6.7)	13 (± 4)	41.3 (± 4)
22/6/01	22.8 (± 3.3)	24.5 (± 8.2)	21.3 (± 6.1)	34.5 (± 2.3)
25/6/01	19 (± 2.1)	18.8 (± 5.6)	25.3 (± 5.1)	48 (± 7.3)
29/6/01	18.5 (± 3.3)	8.3 (± 1.9)	17 (± 3.9)	28.3 (± 7.6)
Mean Total over 12 days	86 (± 3.5)	82.7 (± 14.3)	82.7 (± 15.2)	173 (± 17)

Table 7.2 Furzefield – Mean number of *M. aeneus* per water trap (±SE)

	Blank (Control)	Low lavender	High lavender	2-phenyethyl isothiocyanate
19/6/01	5 (± 1.5)	7.5 (± 1.4)	4.5 (± 1.5)	7.3 (± 1.9)
22/6/01	5.8 (± 1.7)	5.5 (± 2.1)	7 (± 0.9)	13.5 (± 3.1)
25/6/01	10.5 (± 2.8)	10 (± 1.3)	8.5 (± 2.2)	26.3 (± 4.2)
29/6/01	6.25 (± 1.3)	5.5 (± 2.2)	3 (± 0.7)	7.3 (± 1.5)
Mean Total over 11 days	27.5 (± 6.4)	28.5 (± 6.6)	23 (± 3)	54.2 (± 8.8)

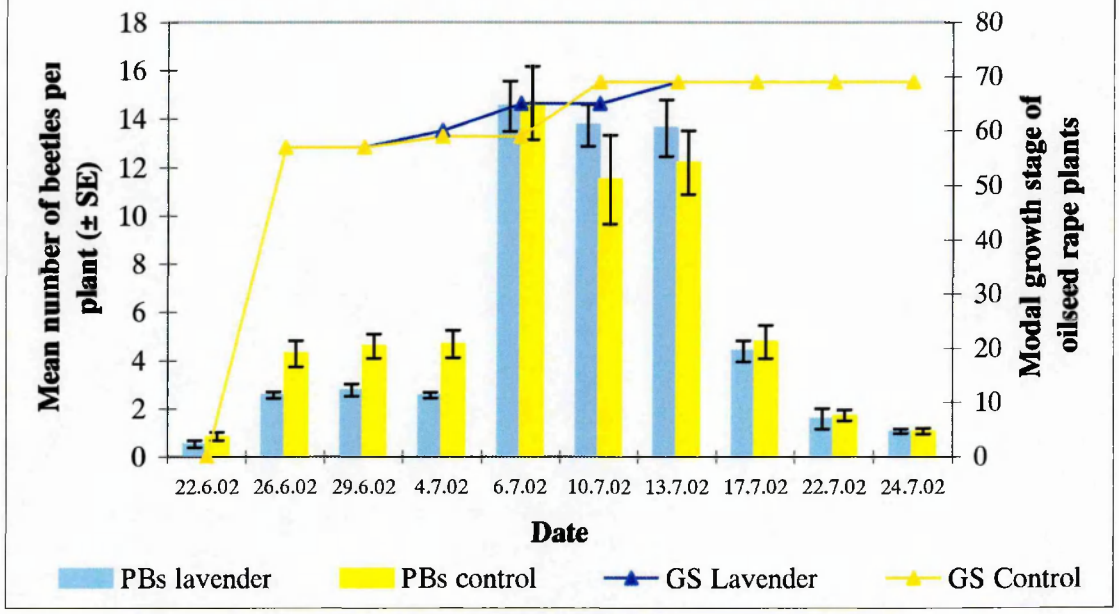
The data were analysed using the totals for each treatment. The two experiments in the different fields were analysed separately. The 2-phenylethyl isothiocyanate treatment was significantly preferred over the other three treatments in both fields (Claycroft $F_{1,6}=278.7$ $p<0.001$ and Furzefield $F_{1,6}=28.4$ $p=0.002$). However the catch in the blank control treatment was not significantly different from the lavender treatments combined (Claycroft $F_{1,6}=0.33$ $p=0.587$ and Furzefield $F_{1,6}=0.1$ $p=0.763$) or from the high lavender treatment alone (Claycroft $p=0.637$ and Furzefield $p=0.509$). The mean catches for the two lavender treatments were not significantly different (Claycroft $F_{1,6}=0.00$ $p=1$ and Furzefield $F_{1,6}=0.74$ $p=0.424$).

7.4.2 Field plot experiment

7.4.2.1 Adult counts

The number of adults in the field plots was sampled from the 22nd June to the 24th July. During that time, the numbers varied between the treatments. From June 26th ($F_{1,14}=9.47$ $p=0.008$) through to July 4th ($F_{1,14}=13.42$ $p=0.003$), there were significantly more pollen beetles on the control-treated plots than the lavender-treated plots (Figure 7.3). On the 6th July, the numbers in both treatments increased rapidly to similar levels ($F_{1,14}=0.00$ $p=0.947$), followed by a period when there was no significant difference between treatments. On the 17th July, the numbers of adult beetles fell to the earlier lower levels and were equally distributed between the two treatments ($F_{1,14}=0.22$ $p=0.643$) and remained so for the rest of the assessments.

Figure 7.3 Number of adult *M. aeneus* beetles per plant (PB) and oilseed rape growth stage (GS) in the field plot experiment



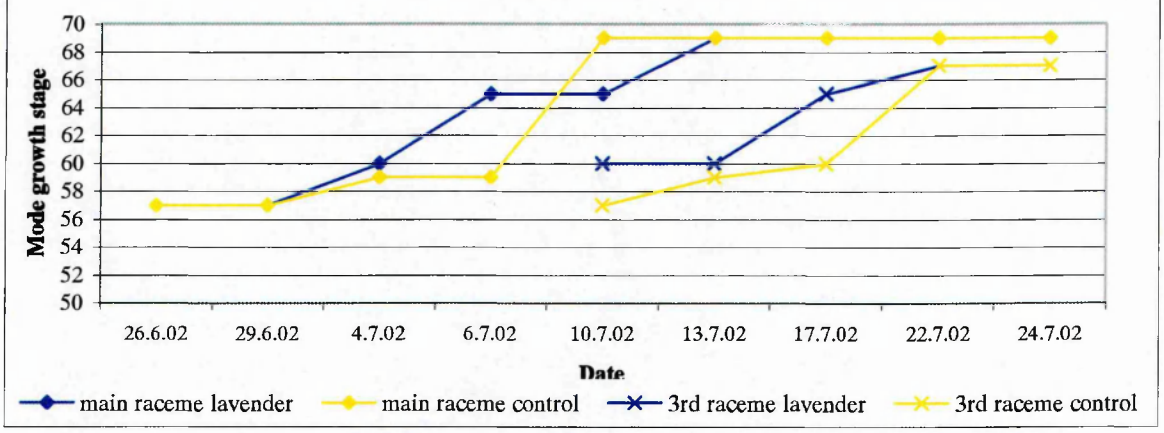
7.4.2.2 Growth stage assessments

At the start of the assessments, the plants in both treatments were at the same growth stage (GS mode = 0 then 57). After the first three assessments, treatment differences emerged. The plants in the lavender-treated plots developed in a typical pattern; developing from green bud GS 51-57, to yellow bud GS 59, through flowering GS 60-67 to petal drop GS 69. However, not all the plants in the control plots flowered. The overall growth stage of the control plots advanced to yellow bud GS 59, and then jumped to petal drop GS 69 (Figure 7.3). The only statistically significant treatment difference was on the 6th July when the plants in the lavender-treated plots were at GS 63 whereas the plants in the control treated plots were at GS 59 (U=2413 p=0.007).

There was visible pollen beetle feeding damage to the plants in the control plots and this was the most likely cause of the differences in growth of the plants between the two treatments. However, due to the numerical code used to record the growth stage, limited variability in growth stage could be distinguished in the records. Therefore, regression analysis into the relationship between beetle numbers on the 4th July and subsequent growth stage treatment differences (6th July) did not show any statistically significant results.

The assessment of the 3rd raceme showed that it was not only the main raceme that showed evidence of treatment differences (Figure 7.4). The 3rd raceme of plants in the lavender treated plots were beginning to flower (GS 60) from the 10th July onwards, whereas the 3rd raceme of the control plots did not flower until a week or two later. This is an indication that the higher amount of insect damage led to extensive changes in the growth pattern of the oilseed rape plants in the control plots.

Figure 7.4 Growth stage mode of main and 3rd racemes in the field plot experiment



7.4.2.3 Bud samples

The bud samples were taken on the 1st July when all the plants in the experiment were at roughly the yellow bud stage (GS 59). There were higher numbers of beetles on the control plots than the lavender plots up to this point but the damage-induced growth stage differences were not evident at this stage (Section 7.4.2.2).

Overall, the total number of buds per raceme ($p=0.011$) and the proportion of undamaged buds ($p=0.008$) were significantly greater for the lavender treatment compared to the control (Table 7.3). However, for buds that showed oviposition damage, the number of eggs and larvae per bud was similar for both treatments ($p=0.247$).

Table 7.3 Results from bud samples

	Lavender treated mean (± SE)	Control treated mean (± SE)	F	p
Total no. of buds per raceme	23.2 (± 0.8)	19.3 (± 1.1)	8.53	0.011
% of buds with damage	76.6 (± 4.7)	91.8 (± 1.5)	9.40	0.008
No. eggs & larvae per damaged bud	2.1 (± 0.11)	2.3 (± 0.09)	1.46	0.247

7.4.2.4 Yield analysis

The overall fresh weights of the plot samples were corrected to dry weights by calculating the proportion of dry matter from a small sample and then adjusting the fresh weight of the full sample. There were no differences between treatment in the dry weight of the samples (p=0.978) (Table 7.4).

Despite overall mass being similar, the architecture of the plants was significantly different between the two treatments (Table 7.4). There was a significant increase in the number of racemes per plant in the control plots compared to the lavender-treated plots (p=0.045). The number of pods per plant was slightly greater for the lavender-treated plots compared to the control plots, however this difference was not statistically significant (p=0.433). However, the main difference was that the number of pods per raceme was significantly greater for the lavender-treated plants compared to the control plants (p=0.003). The weight of 1000 seeds (p=0.115) and the estimated mean number of seeds per plant (p=0.571) were the same for both treatments.

Table 7.4 Results from yield analysis

	Lavender treated mean (± SE)	Control treated mean (± SE)	F	p
Dry weights (kg)	0.22 (± 0.02)	0.22 (± 0.02)	0.00	0.978
No. racemes per plant	11.61 (± 1.1)	14.96 (± 1)	4.83	0.045
No. pods per plant	39.6 (± 4.2)	35.6 (± 2.6)	0.65	0.433
No. pods per raceme	3.82 (± 0.2)	2.71 (± 0.2)	12.52	0.003
Weight 1000 seeds (g)	2.6 (± 0.1)	2.5 (± 0.04)	2.82	0.115
Estimated no. seeds per plant	577.8 (± 87)	519.3 (± 51)	0.34	0.571

7.5 DISCUSSION

Blight & Smart (1999), using coloured water traps in the field, showed that more *M. aeneus* were caught in yellow traps compared traps painted with other colours, but the numbers of insects caught in the yellow traps were enhanced by up to 3 times by the addition of volatiles. They also showed that a trap painted with a colour such as cream or black caught significantly more beetles when an attractive odour was added, although the catch was only enhanced by a small amount. They concluded that visual cues might be more important than olfaction in the orientation of this species. However, the same individual can display a variety of responses to a stimulus depending on the strength of the stimulus relative to other signals available (Aluja & Prokopy, 1993), and the crop was at a range of different flowering stages during Blight & Smart's study.

In the experiments in this chapter, the yellow water traps also caught more insects with the addition of 2-phenylethyl isothiocyanate, in agreement with the findings of Blight & Smart (1999). However, although the addition of some repellent chemicals in the study by Blight & Smart (1999) slightly decreased catches, lavender did not reduce the number of beetles below the control level in the experiments reported in this chapter. The crop was in very early stages of development (pre-green bud), and this could mean that the insects were simply responding to the yellow visual signal. However, the fact that *M. aeneus* can locate oilseed rape in very early bud stage (before any yellow flowers appear) (Ruther & Thiemann, 1997) (personal observation) indicates that colour recognition is not the only mechanism of host location in the early stage of infestation.

I suggest that yellow traps are best employed for monitoring or to discriminate between odours with attractive or non-responsive effect. This is because repellent odours cannot be identified in this way as the trapped insects are responding to a strong visual attractant, in the absence of flowering host plants, which possibly overrides the effect of the repellent odour i.e. the insects are responding to the strongest stimulus available. For this reason, the field-plot experiment is likely to give a more accurate portrayal of the actual effect of lavender odour.

The field-plot experiment was conducted during the early stages of infestation of a spring rape crop. The treatments were applied as the first insects began to arrive in the field. The

lavender essential oil treatment caused a significant reduction in the number of adult *M. aeneus* infesting the plants compared to the control treatment. A 40% reduction in numbers was achieved up to the 6th of July and more importantly, this was achieved while the crop was at the vulnerable green-bud stage (GS 50-57). This reduction in the number of adults caused a visible difference in the phenology of the plants in the two treatments. The feeding and oviposition damage to the rape plants in the control plots was sufficient to prevent the plants from fully flowering. The plants in the control plots were accelerated from yellow bud (GS 59) to end of flowering (GS 69) due to the loss of viable buds. The lavender-treated plants had fewer adult beetles and therefore suffered a reduced attack during the green bud stage. This led to the significantly higher number of buds per raceme for the lavender-treated plants at the yellow bud stage, and subsequently these buds developed into flowers.

An interesting change in adult numbers occurred after the critical green-bud stage. The damaged control plants may have no longer provided the same attraction to the beetles (due to lack of flowers and reduced numbers of buds) and so it is possible that the lavender-treated plants became more attractive than the control plants. Once again, the beetles therefore are displaying plasticity in their choice of host plant according to the strongest signal available (Bernays, 1999).

These data also lend support to earlier findings where the repellent odours are only effective during host location; once the beetle has alighted on a host plant, the repellent is no longer effective (chapter 6). In this field experiment, the 40% reduction in adult numbers only led to a 20% difference in the proportion of undamaged buds in the lavender-treated plots (compared to the control plots). Therefore, there was no evidence that, after landing in the lavender-treated plots, the insects were behaving any differently compared to those in the control plots, as the reduction in damage can simply be attributed to the reduction in adult numbers. Had there been an additional effect on those beetles feeding or ovipositing in the treated plots because of the lavender odour, there would have been more than 40% improvement in proportion of undamaged buds.

Further evidence that non-olfactory stimuli are more important to these insects after landing on a host plant, is that the number of larvae and eggs laid per damaged bud was the same for both treatments. After chewing an oviposition hole in a bud, female *M. aeneus*

place their abdomens over the hole several times before ovipositing (Borg & Ekbom, 1996). There is evidence to suggest that host quality assessment takes place during this behaviour, as fewer oviposition holes are accepted for oviposition on unsuitable host plants (Borg & Ekbom, 1996). However, once accepted for oviposition, the number of eggs laid per bud was similar for both suitable (with pollen) and unsuitable (sterile plants, without pollen) hosts (S. Cook, unpublished data). Since the number of eggs laid per damaged bud is the same for both treatments in this experiment (Table 7.3), it indicates that there was no rejection of the lavender-treated plants during ovipositional host quality assessment behaviour.

Several authors have reported the ability of oilseed rape plants to compensate after attack from pollen beetles (Williams & Free, 1979; Axelsen & Nielsen, 1990). In this experiment, there was evidence that plants in the control plots compensated for the damage caused by the high numbers of pollen beetles. Despite losing buds at the yellow bud stage, by the end of the experiment, the control plot plants had a higher number of racemes per plant. This highly branched development is indicative of the loss of the main growth shoot in plants. Therefore, early season pest damage actually caused a change in the overall architecture of the plants and the use of lavender odour prevented this occurring. However, due to this ability of compensatory growth, the control plants did not show a reduction in plant weight at the time of harvest. The number of seeds and seed weights were similar in both treatments, however they may have been trade-offs between seed production (Daniels *et al.*, 1986) and compensatory growth (Axelsen & Nielsen, 1990). This may also be due to the shorter pod maturation time for the side racemes, leading to lower seed weights and oil content. Compensation for lost buds, especially late in the season, also causes the problem of uneven ripening of pods leading to difficulties at harvest (Winfield, 1986). There could be further deleterious effects on other processes such as decreased resources for defence responses to attack from pathogens or in nutrient assimilation (Cardoza *et al.*, 2002).

More exaggerated treatment differences, and therefore improved yields in the lavender-treated plots, might have been achieved by the use of larger sized field plots. The close proximity of the treatments in this study confounded the results due to the appearance of 'islands' of less damaged plants (in the lavender-treated plots) providing an attractant stimulus within an area of severely damaged plants, thereby encouraging movement of beetles into these plots. This effect could have been predicted from the simulation model as

a repellent-based strategy was shown to work only when the repellent forces the population to move out of the agroecosystem (Potting *et al.*, 2002). However, the additional incorporation of a trap crop would provide a super-attractant stimulus to attract these displaced insects and prevent the invasion of the lavender-treated plots seen in this experiment from the 6th July onwards (Figure 7.3).

The current, heavy reliance on chemical pest control in oilseed rape is leading to the emergence of resistant populations of *M. aeneus* across Europe (Ekbom & Kuusk, 2001; Hansen, 2001) as well as causing mortality in non-targets (Croft, 1990; Cooke, 1993). The push-pull system can combat both of these problems. The use of many elements within the push-pull system will reduce the selection pressure on the pests to evolve resistance mechanisms to any individual component (Pickett *et al.*, 1997). And, the selective use of insecticide (Potting *et al.*, 2002), or better still, the use of specific entomopathogenic micro-organisms (Butt *et al.*, 1998) in the trap crop area will reduce mortality amongst the natural enemies of pests present in the main crop area (Haskell & McEwen, 1998).

Lavender odour still needs to be tested for any effects on beneficial insects such as natural enemies and crop pollinators, however, these results provide useful information on the efficacy of repellents in controlling the pest species, *M. aeneus.* Spring grown oilseed rape is very susceptible to attack from *M. aeneus* because the new generation emerges as the crop is at the vulnerable green bud stage. The insecticide spray threshold for spring rape is an average of 2-3 beetles per plant at any time from very early green bud to yellow bud (ADAS, 1984). In this study, lavender odour kept the level of infestation below this threshold during the critical period of green/yellow bud (22nd June - 4th July, Figure 7.3), whereas the levels in the untreated, control plots were above this threshold. The lavender treatment did reach infestation levels above the threshold, but not until it was flowering and beyond the critical time for pest control. These findings are very encouraging for the future development of a push-pull system.

This chapter forms the final stage of Poppy's experimental sequence in semiochemical research (1991). The investigation of lavender essential oil as a repellent to *M. aeneus* has followed from laboratory bioassays, to semi-field and now field scale. The movement of *M. aeneus* at the next spatial scale, the landscape scale, is investigated in Chapter 8.

CHAPTER 8. FLIGHT OF *MELIGETHES AENEUS* AT A RANGE OF ALTITUDES

8.1 INTRODUCTION

8.1.1 Current knowledge of flight activity of *Meligethes aeneus*

Adult *Meligethes aeneus* fly to flowering plants, on emergence from overwintering, to feed on pollen (Free & Williams, 1978). During this early part of the year, there is a temperature threshold for flight in this species. The lowest temperature for a solitary flight was recorded as 10.2°C (Laska & Kocourek, 1991), however the first gregarious flights (Cooter, 1977; Kenward, 1984) are seen at between 12.3°C and 15°C (Tamir *et al.*, 1967; Tulisalo & Tuomo, 1986; Laska & Kocourek, 1991; Sedivy & Kocourek, 1994). Individual beetles fly to flowering rape crops, firstly winter sown rape (April/May) followed by spring sown crops (June/July) to feed and reproduce. The new generation adults emerge in mid-July and also fly to food sources. Individuals move to feed on other flowering plants once the rape crops have finished flowering and then finally move to overwintering sites.

Pollen beetles are not ground-active as they are rarely caught in pitfall traps, even in rape fields (Warner D. personal communication), therefore, it is assumed that they rely on flight for all dispersal movements. However, all of the movements of individuals described above have been inferred from counts of adults through the year at different sites, not from any direct recording of their flights. Due to their small size, it has proved difficult to track individuals, however some studies have been able to estimate the distances flown. Individuals have been recorded travelling 13.5 km using radioactive tracers (Tamir *et al.*, 1967), but it is likely that they can travel a considerable distance further. Tamir (1967) showed that the insects were able to locate and travel to fields of oilseed rape regardless of wind direction indicating that they used self-powered, directed flights rather than being blown by the wind and were recorded at a distance of 300 m from the release point within two hours of release, although this would be within the boundary layer (see 8.1.2). This is backed-up by the finding of Evans & Allen-Williams (1994) that adult *M. aeneus* used up-wind anemotaxis to locate oilseed rape plants. These studies have shown that *M. aeneus* is capable of sustained, powered flights towards attractant sources, although the greater distance, high altitude, dispersal movements have not been studied.

8.1.2 Boundary layer effect

Insect flight has been divided into those flights that occur within the 'boundary layer' and those that occur outside it. Taylor (1958) suggested the term 'boundary layer' to describe a hypothetical layer of air near the ground within which insects are able to control their movements relative to the ground because their flight speed exceeds wind speed. However, outside of the boundary layer, insects would have to move down-wind as wind speed exceeds their flight speed. The height of this boundary layer varies between species according to size and flight speed, however, it provides a useful representation of the altitudinal profile of insect flight behaviour.

8.1.3 Methodology for studying insect flight

Insect flight has been studied for many taxa at a range of altitudes using a variety of methodologies (Osborne *et al.*, 2002). Ground level flights, within the boundary layer, have been assessed by directly observing flight movements (Kjaerpedersen, 1992) or tracking individuals using radioactive tracers (Tamir *et al.*, 1967) and harmonic radar (Riley *et al.*, 1996; Osborne *et al.*, 1997). Passive collecting methods have also been used to establish flight activity both temporally and spatially. These include water traps (Laska & Kocourek, 1991; Sedivy & Kocourek, 1994), malaise traps (Murchie *et al.*, 2001), window traps (Boiteau *et al.*, 1999) and flight interception traps (Jessop & Hammond, 1993). Attraction traps include light traps (Woiwod & Harrington, 1994), colour and/or odour baited traps (Smart & Blight, 2000). Field counts can also provide information on the patterns of movement of populations (Williams & Free, 1978).

Insect flights have been studied using suction traps (Johnson & Taylor, 1955; Taylor, 1974). These traps collect insects at random by sampling air at a known rate. Two heights of suction trap are currently in use as part of the Rothamsted Insect Survey; 5 ft (1.5 m) and 40 ft (12.2 m) and these can collect samples of insects in all weathers and thereby provide a constant, easily comparable measure of insect density at the two heights.

High-altitude flights have been studied using radar since 1976 (Schaefer, 1976). However a recent development in the technology has enabled the technique to be used for routine, long-term monitoring of aerial migration (Smith *et al.*, 1993). Vertical-looking radar records information about over-flying insects that is related to their speed, direction of movement, orientation, size and shape. Sampling of the aerial fauna at similar heights is

required to calibrate these records for individual species and this has been achieved using a balloon-supported net, which samples the aerial fauna between 180-200 m above ground level (Chapman *et al.*, 2002).

The migratory flights of *M. aeneus* outside of the boundary layer have not yet been studied and therefore the experimental work in this chapter is aimed at characterising this important part of their ecology. This was approached using a novel combination of techniques; field counts, suction traps and vertical-looking radar to provide information on movements both at the ground level and at a range of altitudes.

8.1.4 Novel combinations of methodologies

The aim of this work is to develop an understanding of the importance of flight at different altitudes in the ecology of *M. aeneus* using a novel combination of data from suction traps, VLR and field counts. For the push-pull strategy to be effective, there is a need to understand pest population dynamics at the landscape scale. Spatial information can be extracted, giving a profile of the insects, which can be used to explain the resultant distribution of insects immigrating to oilseed rape fields. For example, reliance on short, self-powered flights at low altitudes is likely to result in an edge-distribution of insects in an oilseed rape field (Murchie *et al.*, 1999; Ferguson *et al.*, 2000), whereas higher altitude flights are more likely to result in a scattered distribution in the field due to the different angle of incoming flights. Temporal information can also be extracted from such data and used to identify seasonal patterns of flight and thereby timings of pest outbreaks on the oilseed rape crops (Woiwod, 1991). The data can also be linked with meteorological data and mathematical models used to forecast likely population dynamics (Masterman *et al.*, 1996). All of this information will be of great value in terms of timing the application of the different semiochemical-based control methods within the push-pull strategy.

8.2 AIMS

1. To characterise the phenology of winter and spring-sown oilseed rape crops and correlate these with abundance of *M. aeneus* on the plants.
2. To identify diurnal patterns of flight movements of *M. aeneus* and their use of flight at a range of altitudes throughout their active season.
3. To identify meteorological factors influencing flight at different altitudes by these pests.
4. To link the flight patterns with the crop phenology to enable predictions of the timing of, and possible triggers for, crop immigrations by *M. aeneus.*

8.3 MATERIALS AND METHODS

The data for this experiment were collected from March to August in 2001 and 2002. In order to compare the data sets, the data were summarised into weekly values.

8.3.1 Field assessments

Weekly assessments of populations of *M. aeneus* were conducted over two years in fields of oilseed rape on Rothamsted Farm (Appendix 2). In 2001, five fields were sampled from March to July. The winter oilseed rape fields were Meadow, White Horse I and Furzefield. The spring oilseed rape fields were Claycroft and Furzefield. In 2002, four fields were sampled. The winter oilseed rape fields were New Zealand, Highfield and Sawyers II, whilst only one spring oilseed rape field, Great Harpenden, was available.

Four 60 m linear transects were sampled in each field. All starting at the edge of the crop and running into the centre of the field. One transect was walked from each side of the field. Every 3 m along each transect, an oilseed rape plant was sampled and a record made of the growth stage (Table 2.3) and the number of *M. aeneus* adults present. Early in the 2001 season, 20 plants per transect were assessed, but this was later reduced to 10 plants at 6 m distances due to time constraints.

The number of plants sampled and beetle counts taken each week (N) varied depending on the weather conditions. Counts and growth stage data were collected on days without heavy rain, as the insects were less visible (i.e. inside the flowers) in rainy conditions. Therefore, there were weeks when there was only time to sample one field before the onset

of rainy weather. The week numbers and their corresponding N values are shown in Table 8.1.

Table 8.1 Numbers of samples of plant growth stage and beetle counts (N) for winter rape (WR) and spring rape (SR)

2001			2002		
Week	Dates	N	Week	Dates	N
1	12-18 March	240 (WR)	1	4-10 March	120 (WR)
2	19-25 March	240 (WR)	2	11-17 March	120 (WR)
3	26/3-1 April	240 (WR)	3	18-24 March	120 (WR)
4	2-8 April	240 (WR)	4	25-31 March	120 (WR)
5	9-15 April	240 (WR)	5	1-7 April	120 (WR)
6	16-22 April	240 (WR)	6	8-14 April	120 (WR)
7	23-29 April	40 (WR)	7	15-21 April	120 (WR)
8	30/4-6 May	240 (WR)	8	22-28 April	120 (WR)
9	7-13 May	240 (WR)	9	29/4-5 May	120 (WR)
10	14-20 May	160 (WR)	10	6-12 May	80 (WR)
11	21-27 May	120 (WR)	11	13-19 May	120 (WR)
12	28/5-3 June	120 (WR)	12	20-26 May	120 (WR)
13	4-10 June	120 (WR)	13	27/5-2 June	120 (WR)
14	11-17 June	120 (WR)	14	3-9 June	
15	18-24 June	80 (WR) 40 (SR)	15	10-16 June	40 (SR)
16	25/6-1 July	80 (WR) 40 (SR)	16	17-23 June	40 (SR)
17	2-8 July	80 (WR) 40 (SR)	17	24-30 June	40 (SR)
18	9-15 July	60 (SR)	18	1-7 July	40 (SR)
19	16-22 July	60 (SR)	19	8-14 July	40 (SR)
20	23-29 July	60 (SR)	20	15-21 July	40 (SR)
21	30/7-5 August	20 (SR)	21	22-28 July	40 (SR)
22	6-12 August	20 (SR)	22	29/7-4 August	
23	13-19 August	20 (SR)	23	5-11 August	
24	20-26 August	20 (SR)	24	12-18 August	
25			25	19-25 August	

8.3.2 Suction traps

The aerial density of *M. aeneus* was measured using the Rothamsted Insect Survey suction traps (Macaulay *et al.*, 1988) (Figures 8.1 & 8.2, Appendix 2). The density was measured at two heights; 12 m and 1.5 m. Both traps were run continuously and the samples collected daily. The samples were stored in 70% ethanol with glycerol in sealed glass vials. Samples were taken from March to the end of August in 2001 and 2002 and the number of *M. aeneus* recorded.

The airflow through both suction traps is ~ 50 m^3 per minute for the 12 m trap and ~ 9 m^3 per minute for the 1.5 m trap (Wright S. unpublished data). The daily catch (restricted to 12 hours i.e. daytime) of insects was converted to aerial density in 10^3 m^3 air according to the following equations;

1.5 m trap = (9 x 60 minutes x 12 hours) m^3 = 6,480 m^3 air sampled per day

$\therefore$ x insects/6.48 = density of x in 10^3 m^3 air

12 m trap = (50 x 60 minutes x 12 hours) m^3 = 36,000 m^3 air sampled per day

$\therefore$ x insects/36 = density of x in 10^3 m^3 air

Figure 8.1 1.5 m and 12 m suction traps at Rothamsted (see Appendix 2)

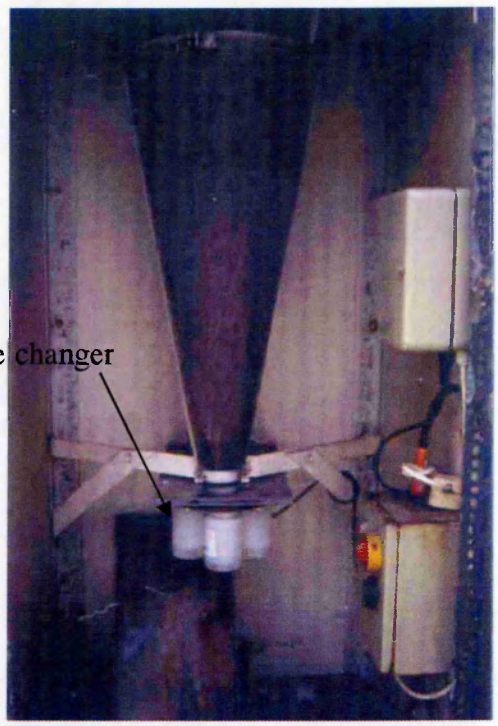


Figure 8.2 Internal construction of the 12 m suction trap showing bottle changer

8.3.3 Vertical-looking radar

8.3.3.1 Background information

Vertical-looking radar (VLR) is a more recently developed technique specifically intended to routinely monitor the flight behaviour of migrant insects (Smith *et al.*, 1993). The VLR is sited on the top of a building at Rothamsted Research (Appendix 2) and has been operating since 1999. Overflying insects modulate the radar signal in a way that is related to their speed and direction of movement, their orientation, size and shape. The species of

insect cannot be determined using this method alone, therefore these continual recordings can be restricted to specific times during the day and to specific insect weights to reduce the 'noise' from other flying insects.

8.3.3.2 Weight of adult M. aeneus *individuals*

Fifteen mixed sex, adult *Meligethes aeneus* were live-trapped in the 12 m suction trap, killed by freezing at -20°C and weighed. The mean weight was 1.52 mg (±0.04) ranging from 1.24 to 1.8 mg. Therefore, it was assumed that the majority of *M. aeneus* individuals were included in the 1-2 mg weight category of the VLR data. However, many other day-flying insects were also trapped, killed and weighed in the same manner and these also fell into the 1-2 mg weight range. Therefore, it cannot be easily estimated what proportion of the radar records are composed of *M. aeneus*, but peaks can be corroborated with the suction trap data at 1.5 m and 12.2 m and the assumption was made that the majority of the co-occurring peaks consisted of *M. aeneus*.

8.3.3.3 VLR data collection

As *M. aeneus* weigh between 1 and 2 mg, the radar can only detect this size of insect at the lowest sampling band of 150-195 m above ground level. The amplitudes of any signals captured within that range gate were recorded for a 5-minute period every 15 minutes, 24 hours a day. Various parameters are automatically determined and recorded. The aerial density of overflying insects is found by calculating the volume sensed by the VLR for every target and are expressed in terms of the mean number of insects per 10^7 m^3 as calculated for each 5-minute period.

Dr J. W. Chapman collected and presented the radar records filtered to daytime records of targets between 1-2 mg from March to end of August in 2001 and 2002. The aerial densities were then summed to provide the daily density of correct-sized targets per 10^7 m^3 and then corrected to density in 10^3 m^3 air to be comparable with the suction trap density data.

8.3.3.4 Aerial netting

In order to establish exactly which insect species are flying at altitude, flying insects were collected using aerial netting (Chapman *et al.*, 2002). A net was suspended from a balloon flying at 180-200 m (the same height as the records from the radar). The samples were

collected at Cardington airfield, U.K. on several dates in July of 1999, 2000 and 2002 at a variety of times during the day, while weather conditions permitted. These data were collected and used by Dr Chapman to confirm that *M. aeneus* was recorded flying at this altitude.

8.3.4 Diurnal flight activity

A second 12 m suction trap was operated through the summer of 2001 to investigate the diurnal flight activity of *M. aeneus*. The trap was operated from 19th May to 23rd August. Insect samples from four time periods were collected separately using a timed bottle changer attached to the suction trap (times shown in BST):

'Dawn' 06:00-08:00

'Day' 08:00-18:00

'Dusk' 18:00-20:00

'Night' 20:00-06:00

8.3.5 Meteorological data

Meteorological data were collected from the Rothamsted meteorological station sited within 20 m of the suction traps (Appendix 2). The data were collected every 15 minutes, 24 hours a day. The data were combined into daily or weekly means. The variables used were:

Maximum temperature (°C)

Minimum temperature (°C)

Mean temperature (°C)

Mean solar radiation (W/m^2)

Mean wind speed (m/s)

Total rainfall (mm)

Mean relative humidity (%)

8.3.6 Data presentation and statistical analysis

The data were summarised into weekly values to enable comparisons between data sets and between years.

8.3.6.1 Characterisation of the phenology of the oilseed rape crop and its correlation with population counts of M. aeneus *on the plants*

The modal value for the growth stage of winter and spring oilseed rape (Table 2.3) in 2001 and 2002 was calculated weekly across all fields (Table 8.1) and is represented using a colour code (Figure 8.3).

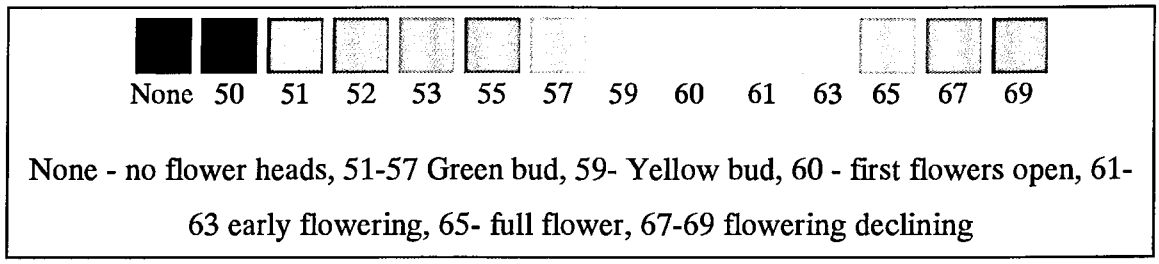


Figure 8.3 Colour code for growth stages of oilseed rape (Lancashire *et al.*, 1991)

Temporal distribution of *M. aeneus* on rape plants

The mean number of pollen beetles per plant was calculated weekly across all the fields for 2001 and 2002. The proportion of the crop in flower (GS 60-65) each week was calculated and used to investigate the temporal relationship between the numbers of *M. aeneus* and the phenology of the oilseed rape plants. The mean number of *M. aeneus* per plant was compared with the proportion of the crop in flower for all plants each week in each year for both winter and spring rape by calculating Spearman's rank correlation coefficients. The significance of the correlation between these variables was determined by calculating the Student's t approximation.

Spatial distribution of *M. aeneus* on rape plants

The spatial distribution of *M. aeneus* was mapped using Surfer (Golden software, Inc. Version 7, 1999) using x,y co-ordinates for each sampling position within the field. Classed post maps were produced for the first date after colonisation, with darker colours representing higher densities of pollen beetles per position. The maps were produced as a representation of the distribution and are not shown to scale, as the sizes and shapes of the fields varied. The spring rape fields were too small to sample along the same layout of transects and they often had to over-lap, therefore Claycroft in 2001 was the only spring rape field mapped for spatial pattern. No further analysis of spatial pattern was conducted.

8.3.6.2 Identification of patterns of flight movements of **M. aeneus** *throughout their active season; use of flight at different altitudes*

Seasonal patterns of flight at altitude were presented graphically as weekly densities of insects recorded at the three altitudes for 2001 and 2002. Visual comparisons are made across the season. The influence of meteorological factors was investigated in Section 8.4.3.

8.3.6.3 Identification of meteorological factors influencing flight at different altitudes

Daily meteorological values and beetle densities at the three altitudes were collated for 2001 and 2002. Spearman's rank correlation coefficients were calculated for comparisons between all the variables and the results presented in a symmetric matrix. The significance of the correlation between each pair of variables was determined by calculating the Student's t approximation. This non-parametric, rank-order test was used, as there was strong autocorrelation between the meteorological variables.

8.3.6.4 Linkage of the flight patterns with the crop phenology to enable predictions of the timing and possible triggers for crop immigrations by **M. aeneus**

For each field, the weekly data were divided into periods of immigration and emigration. Immigration was defined as those weeks leading up to and including the maximum number of beetles per plant. These data were combined with the modal growth stage of the crop in each field and the proportion of plants in flower (GS 60-65) for each week. These variables were then compared with the previous week's values for the meteorological variables and insect densities at 1.5, 12 and 200 m and correlation matrices were produced using Spearman's rank correlation coefficients. Emigration was defined as the period following the maximum number of beetles per plant in each field and the data for these weeks were compared with the same week's meteorological variables and insect densities, again using Spearman's rank correlation coefficients.

8.4 RESULTS

8.4.1 Characterisation of the phenology of the oilseed rape crop and its correlation with population counts of *M. aeneus* on the plants

8.4.1.1 Temporal distribution of M. aeneus *on rape plants*

The oilseed rape crops developed in a similar manner in the two years with the winter rape showing a longer development time than spring-sown rape (Figure 8.4a and 8.5a). The mean number of *M. aeneus* beetles per oilseed rape plant from all the fields combined (see Table 8.1) was calculated weekly and plotted for 2001 (Figure 8.4a) and 2002 (Figure 8.5a) on both winter and spring oilseed rape.

In both years, there was a similar pattern of colonisation by *M. aeneus* in the winter and spring rape crops. The first few individuals were present in the winter crop from early March (week 1 in 2001 and week 2 in 2002) when the crop was in early green bud GS 50-51. However, the main population did not arrive until April (weeks 5&6 2002; week 5 2001) when the crop was approaching flowering (GS 65). The numbers dropped on the winter rape through May and June (weeks 12 to 14) as the winter crop finished flowering (GS 67-69). As the new generation of *M. aeneus* emerged in early July, the population reached its annual peak in the spring crop at week 17 in 2001 when the crop was in full flower (GS65), but occurred later in 2002 (week 19) when the crop was in yellow bud (GS 59) due to late establishment of the crop. The population again tailed off as the crop finished flowering (GS 67-69).

The mean number of beetles per plant increased as the crop progressed towards flowering for both winter and spring crops, reaching a peak during flowering (GS 65), after which the number of beetles decreased again (Figure 8.4a & 8.5a). This relationship was investigated by comparing the weekly means for the proportion of the crop in flower against the mean number of beetles per plant for winter and spring crops across both years. The Spearman's rank correlation analysis showed that for winter rape, the proportion of the crop in flower was significantly correlated with the mean number of beetles per plant in both 2001 (r_s=0.629 df=15 p=0.007) and 2002 (r_s=0.725 df=11 p=0.005). However, although there was a similar relationship between these variables in spring rape in 2001 (r_s=0.533 df=8

p=0.115), this was not significant due to the lower number of replicates. There were too few replicates in 2002 to conduct the Spearman's rank correlation test.

There was a time-gap between the end of the winter rape flowering and the beginning of the spring rape flowering. Therefore, the pollen beetles require other host plants to feed during this time and are moving on a landscape scale to locate host plants at the required phenological stage i.e. with food and oviposition sites, in response to the decline of these factors in their current location.

8.4.1.2 Spatial distribution of M. aeneus *on rape plants*

The spatial distribution of *M. aeneus* within rape fields during the early stages of immigration was mapped for the fields studied in this chapter (Figures 8.6-8.12). There was no evidence of a strong spatial pattern in any of the fields. White Horse I showed a complete absence of beetles in one area of the field, but this was due to severe pigeon damage to the plants in this area. The fields ranged in size from 1.1 ha to 9.44 ha however no edge effect was seen in any of the fields.

8.4.2 Identification of patterns of flight movements of *M. aeneus* throughout their active season; diurnal patterns and use of flight at different altitudes

8.4.2.1 Diurnal activity

The diurnal activity of *M. aeneus* was studied using a 12.2 m suction trap collecting dawn, day, dusk and night samples in 2001. The total numbers of beetles caught throughout the four-month sampling period are shown in Table 8.2.

Table 8.2 Diurnal activity of *M. aeneus*; total numbers caught in different time periods at 12.2 m in 2001

Sampling period	Times	Total *M. aeneus* caught	Total *M. aeneus* caught per sampling hour
Dawn	06:00-08:00	3	1.5
Day	08:00-18:00	211	21.1
Dusk	18:00-20:00	18	9
Night	20:00-06:00	5	0.5

These results indicate that flight occurs predominantly during the daytime in this species, which concurs with the findings of Lewis & Taylor (1965), who showed that the peak flight time in this species was 12.44 GMT.

8.4.2.2 Seasonal patterns of flight at various altitudes

Together these techniques have provided evidence, for the first time, that *M. aeneus* utilises flight at a range of altitudes, up to the highest recordings of 200 m. The total number of *M. aeneus* caught throughout the season in the suction traps was 136 in the 1.5 m trap and 237 in the 12 m trap in 2001. Much higher numbers were caught in 2002; 504 in the 1.5 m trap and 414 in the 12m trap. From the VLR there were 1916 records of 1-2 mg insects in 2001 and 1500 records in 2002, a proportion of which were *M. aeneus*.

Aerial netting samples from 1999 collected a total of 15 *M. aeneus* in 7 of the 9 sampling days. Aerial netting in 2000 caught a total of 41 *M. aeneus* during 8 of the 11 sampling days (Chapman, J. W. unpublished data). Due to the difficulty in identifying the insect species from the VLR records, from now on, it was thought reasonable to consider that many of the VLR records of 1-2 mg insects were *M. aeneus* because similar methodology has been applied to other VLR studies (Chapman *et al.*, 2002).

The density of *M. aeneus* at 1.5 m, 12 m and 200 m was calculated weekly from March to August in 2001 (Figure 8.4b) and 2002 (Figure 8.5b). Both years show similar patterns. The highest density of beetles occurs at 1.5 m whereas the 12 m and 200 m densities are approximately one order of magnitude smaller.

Flight at different altitudes varies through the season. Early in the season (March-early April, weeks 2-5) there was a predominance of flight at 12 m followed by a period (May through June, weeks 8-16) where flights occurred at all altitudes. However, in July (weeks 17-20) there was the highest density of beetles flying at any height throughout the season, with by far the most at the lowest altitude, 1.5 m. In July there was also a high proportion of flights at 12 m. In August (weeks 21-24) there was a shift towards high altitude flight (at 200 m) and the lower altitude flights tailed off rapidly. This seasonal variation is analysed further in Section 8.4.3.

8.4.3 Identification of meteorological factors influencing flight at different altitudes

Weekly mean temperature, rainfall and wind speed were calculated for 2001 (Figure 8.4c) and 2002 (Figure 8.5c) and visually compared with insect densities at 1.5 m, 12 m and 200 m. In addition, a correlation matrix was formed using daily values of all meteorological variables and insect densities from both years (Table 8.3). The results show that insect density at 12 m is not strongly correlated with time (week) ($p=0.248$) i.e. there is non-linear variability over the season. Density at 200 m is most strongly correlated with an increase in time over the season ($p<0.001$), which is explained by the higher number of flights at high altitude at the end of the season. Insect density at all altitudes is positively correlated with temperature and radiation while negatively correlated with rainfall and wind speed. However, these relationships change at different times of year and this is discussed further in Section 8.4.4.

8.4.4 Linkage of the flight patterns with the crop phenology to enable predictions of the timing and possible triggers for crop immigrations by *M. aeneus*

Correlation matrices were formed for the period of immigration (Table 8.4) and emigration (Table 8.5) into and out of winter rape. The data for the number of beetles per plant in the field were compared with the previous week's data for insect density at altitude and meteorological factors during immigration and the same week's data for emigration. These comparisons were chosen to model the movement of insects between flight and the field. This was repeated for immigration (Table 8.6) and emigration (Table 8.7) into and out of spring rape.

By focusing on the weeks around the initial colonisation of winter and spring crops in each year (Figures 8.4b & 8.5b and Tables 8.4 & 8.6), it is clear that daily patterns of flight are different for the two colonisation periods. Winter rape colonisation is characterised by low insect densities at 1.5 and 12 m over several weeks resulting in a gradual build-up of numbers in the field. There was a statistically significant, positive correlation between numbers of beetles in the fields and insect densities at 1.5 m ($r_s=0.638$ $df=38$ $p<0.001$), 12 m ($r_s=0.563$ $df=38$ $p<0.001$) and 200 m ($r_s=0.604$ $df=38$ $p<0.001$) from the previous week. The first flights follow an increase in daily mean temperatures ($r_s=0.618$ $df=38$ $p<0.001$) and radiation ($r_s=0.826$ $df=38$ $p<0.001$) with low rainfall ($r_s=-0.355$ $df=38$ $p=0.025$).

Spring rape colonisation is characterised by a sharp increase in insect densities at 1.5 and 12 m in early July (week 17 in 2001 and weeks 18-19 in 2002) associated with a rapid immigration into the fields. The number of beetles in the field were statistically significantly, positively correlated with the proportion of the crop in flower (r_s=0.792 df=7 p<0.001) and insect densities at 1.5 m (r_s=0.669 df=7 p<0.001) and 12 m (r_s=0.681 df= p<0.001). Specific meteorological triggers are difficult to identify, and the rapid increase in numbers is most likely to be the emergence of the new generation.

Emigration is less well correlated with insect aerial density. As the number of beetles in winter rape fields declines (seen by the negative correlations with week as the time factor), there is a correlation with insect density at 200 m (r_s=0.475 df=38 p=0.002), but not with densities at any of the other altitudes, while emigration from spring rape is correlated with insect density at 1.5 m (r_s=0.741 df=9 p=0.009).

Overall, the crop phenology (characterised in this analysis by the proportion of the crop in flower) seems to be the most important factor in determining the number of beetles on the crop and the number of flights at the range of altitudes studied. The meteorological factors have less impact over the season than the crop growth stage, which therefore seems to be the best forecasting cue. With only two years data, it is difficult to formulate predictive cues for subsequent colonisation, however, in both years the spring rape was subject to higher numbers of beetles than the winter rape. Also, in both years the highest number of new generation beetles making flights at low altitude and subsequently colonising the nearby crops were in early July. Therefore timing the sowing of the spring crop to ensure the vulnerable green bud stage does not coincide with this emergence would provide an efficient means to reduce pest damage at this crucial point in development.

Figure 8.4a Weekly mean number of *M. aeneus* per plant on winter (▲) and spring (x) oilseed rape 2001. Growth stage of winter (WOSR) and spring rape (SOSR) shown along the bottom (see Figure 8.3).

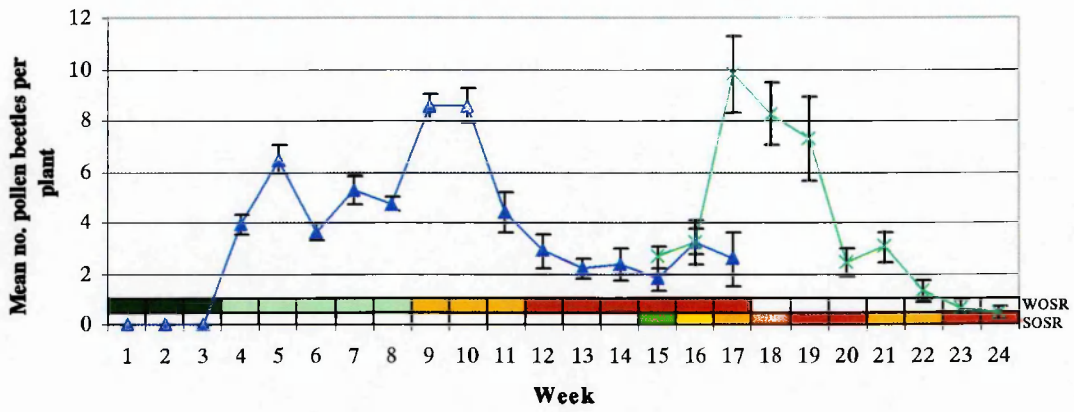


Figure 8.4b Suction trap and VLR weekly densities of *M. aeneus* 2001

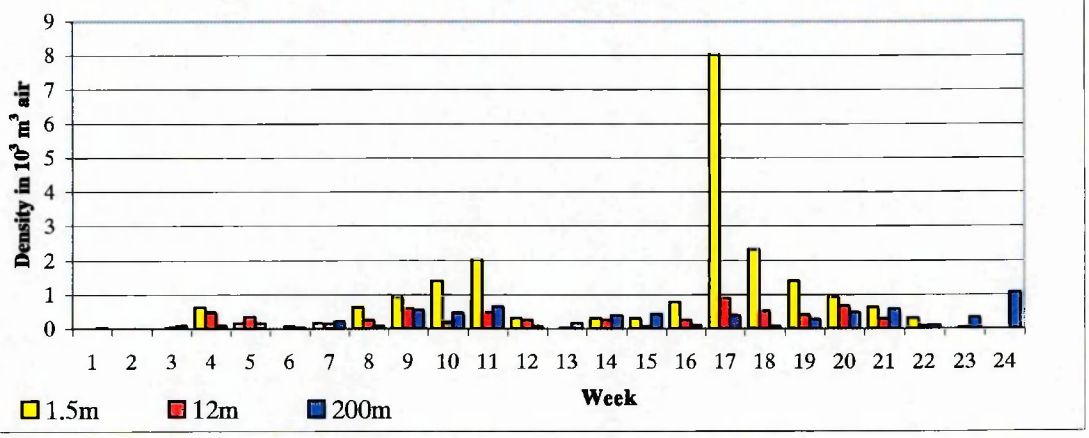


Figure 8.4c Weekly meteorological means 2001

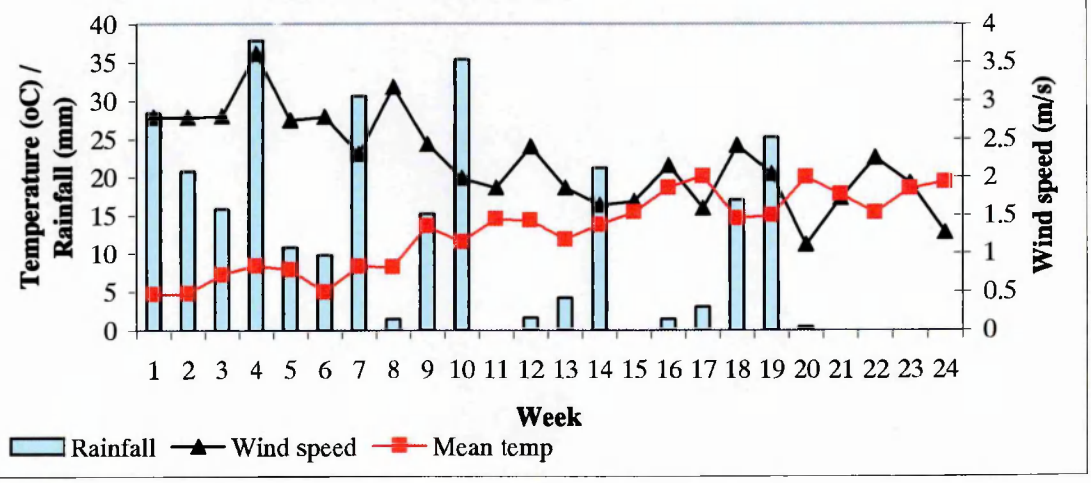


Figure 8.5a Weekly mean number of *M. aeneus* per plant on winter (▲) and spring (x) oilseed rape 2002. Growth stage of winter (WOSR) and spring rape (SOSR) shown along the bottom (see Figure 8.3).

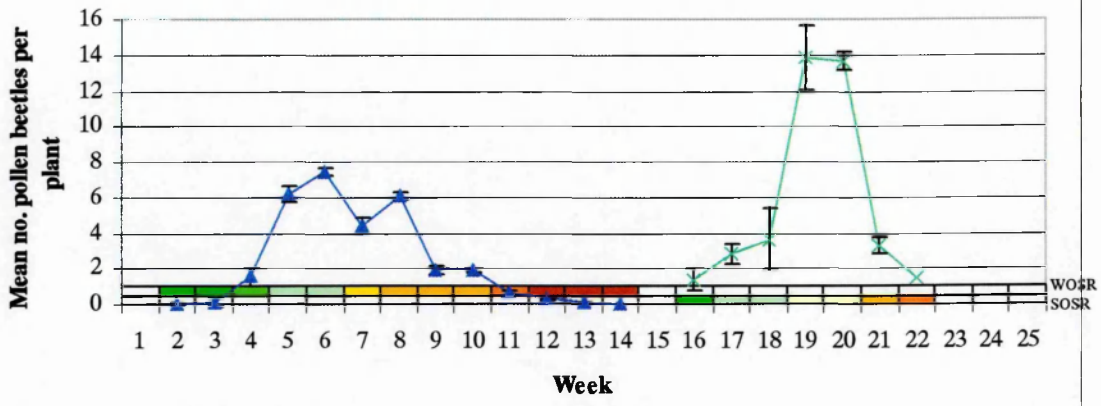


Figure 8.5b Suction trap and VLR weekly densities of *M. aeneus* 2002

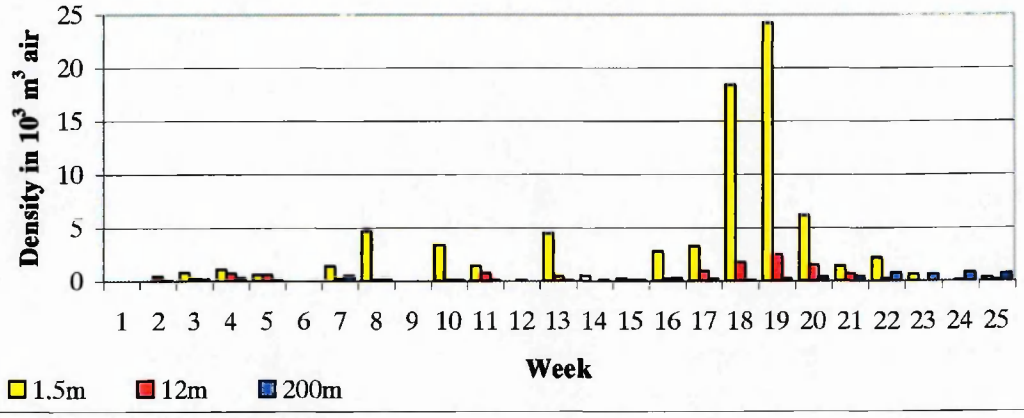


Figure 8.5c Weekly meteorological means 2002

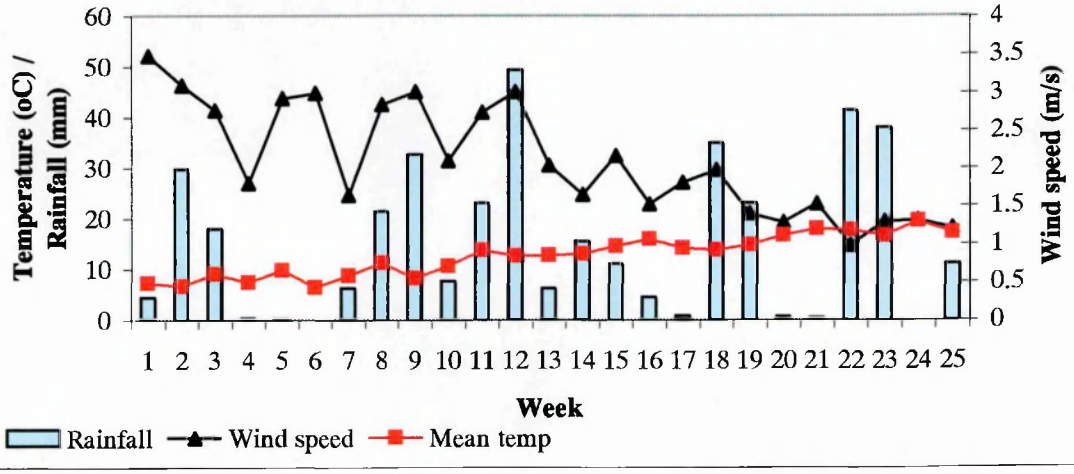


Figure 8.6 Spatial distribution of pollen beetles Winter rape Furzefield 2.4.01

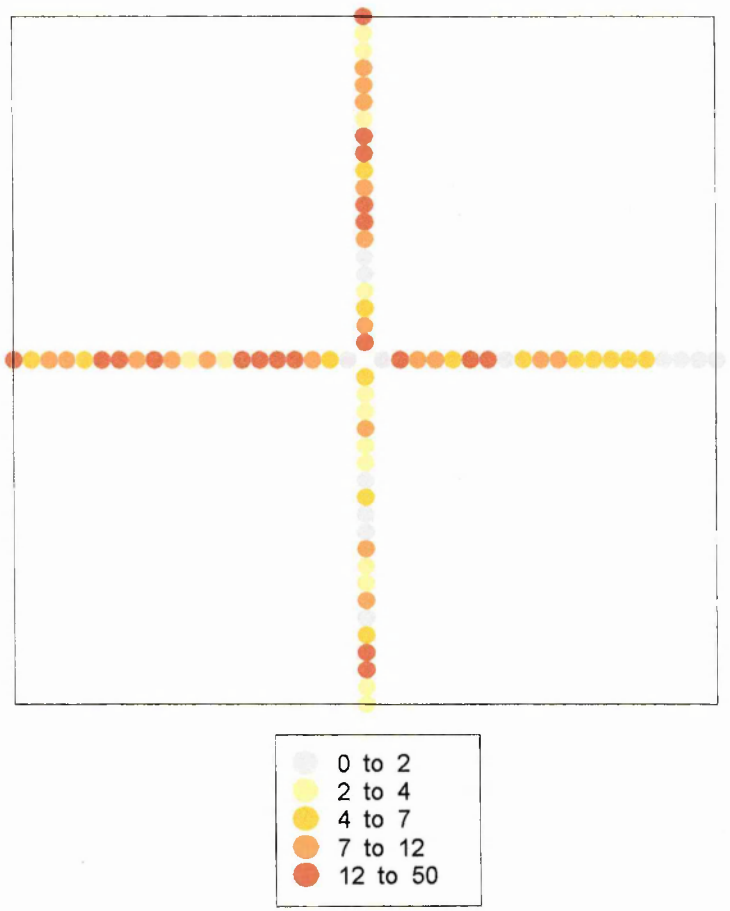


Figure 8.7 Spatial distribution of pollen beetles Winter rape Meadow 2.4.01

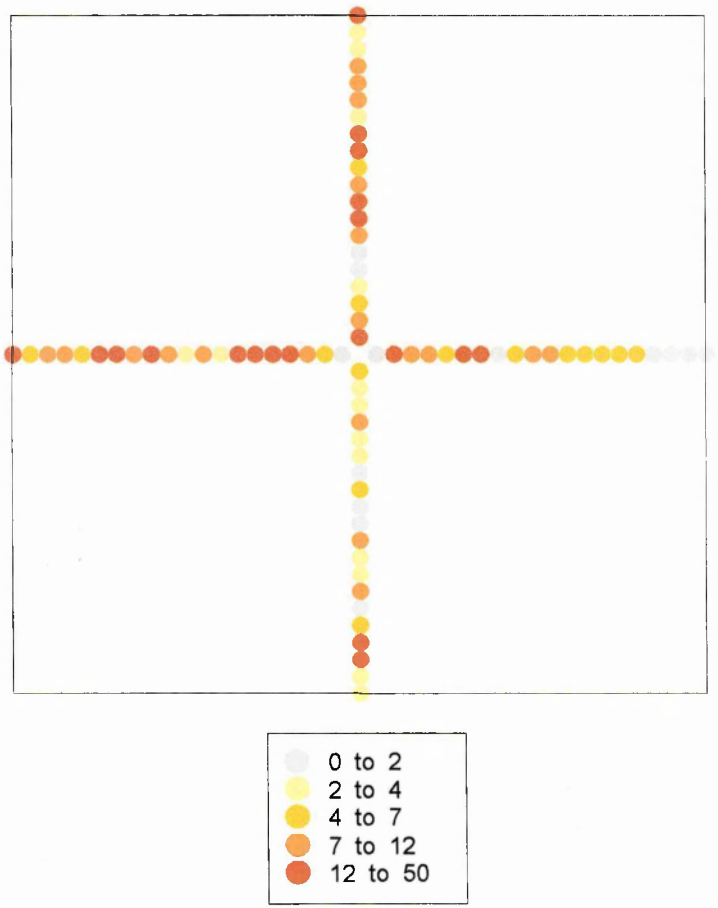


Figure 8.8 Spatial distribution of pollen beetles Winter rape White Horse 2.4.01

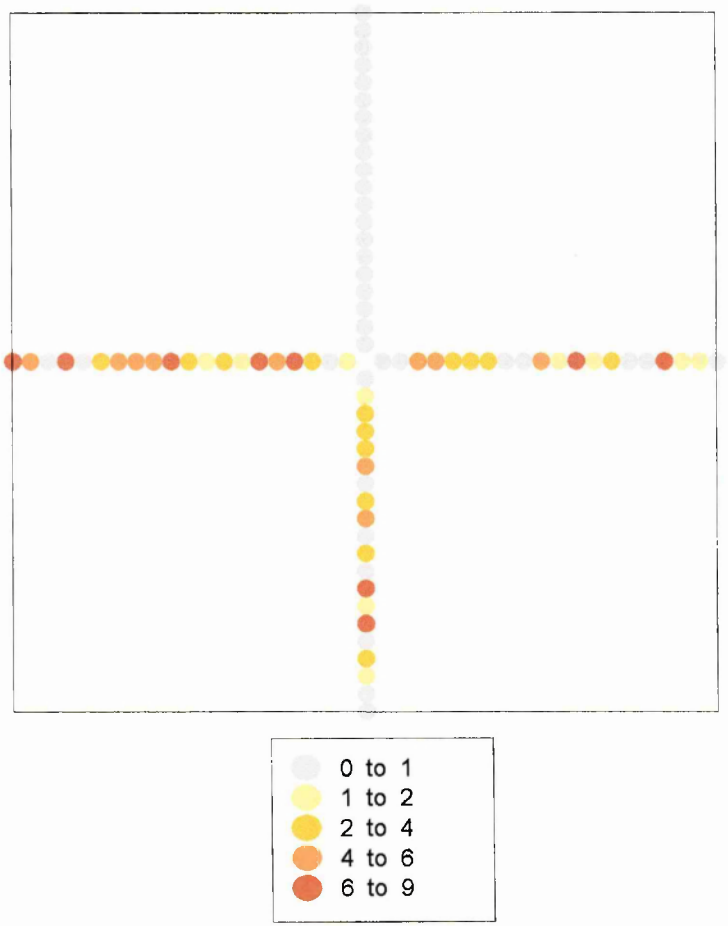


Figure 8.9 Spatial distribution of pollen beetles Winter rape New Zealand 25.3.02

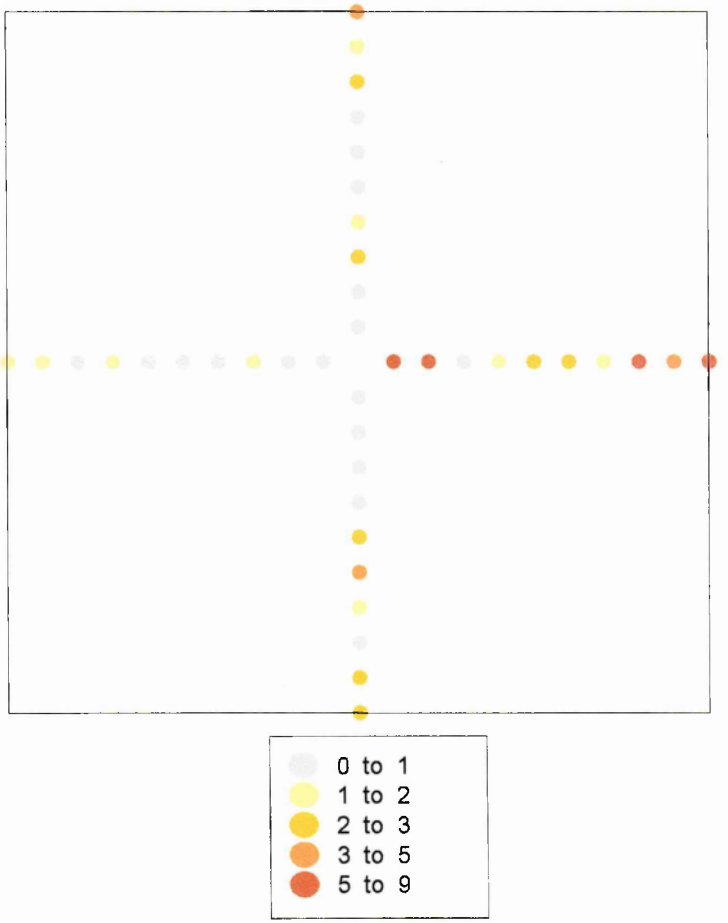


Figure 8.10 Spatial distribution of pollen beetles Winter rape Highfield 25.3.02

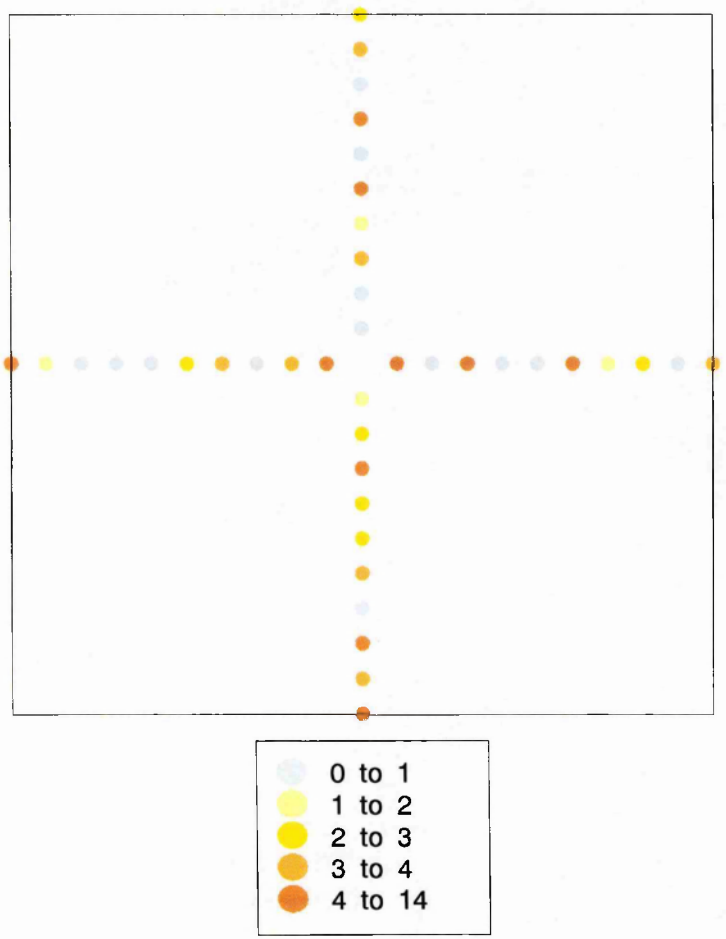


Figure 8.11 Spatial disrtibution of pollen beetles Winter rape Sawyers II 25.3.02

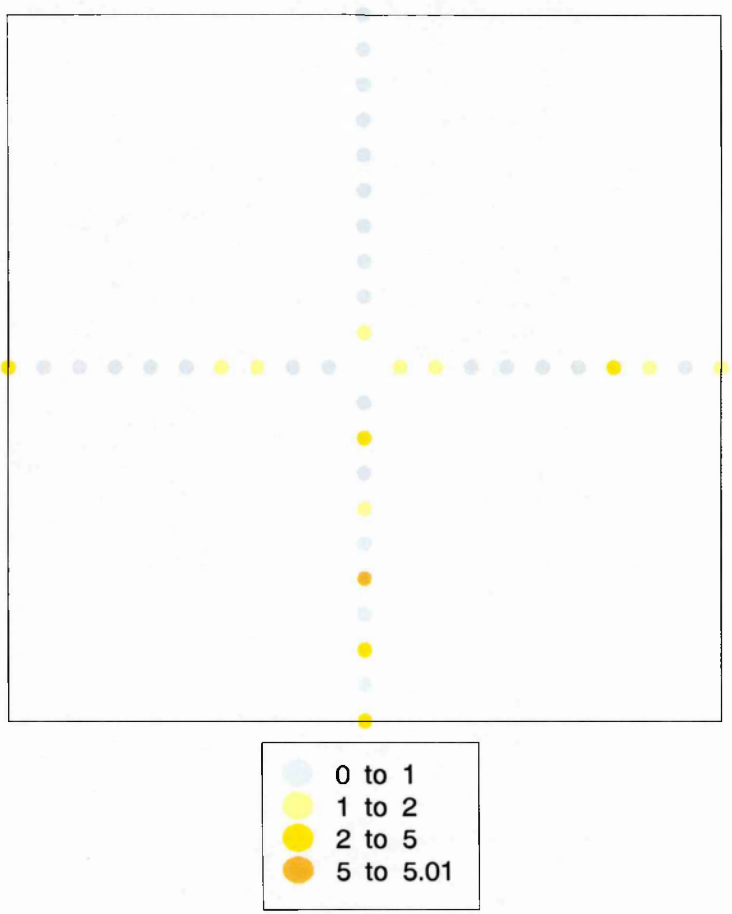


Figure 8.12 Spatial distribution of pollen beetles
Spring rape Claycroft 18.6.01

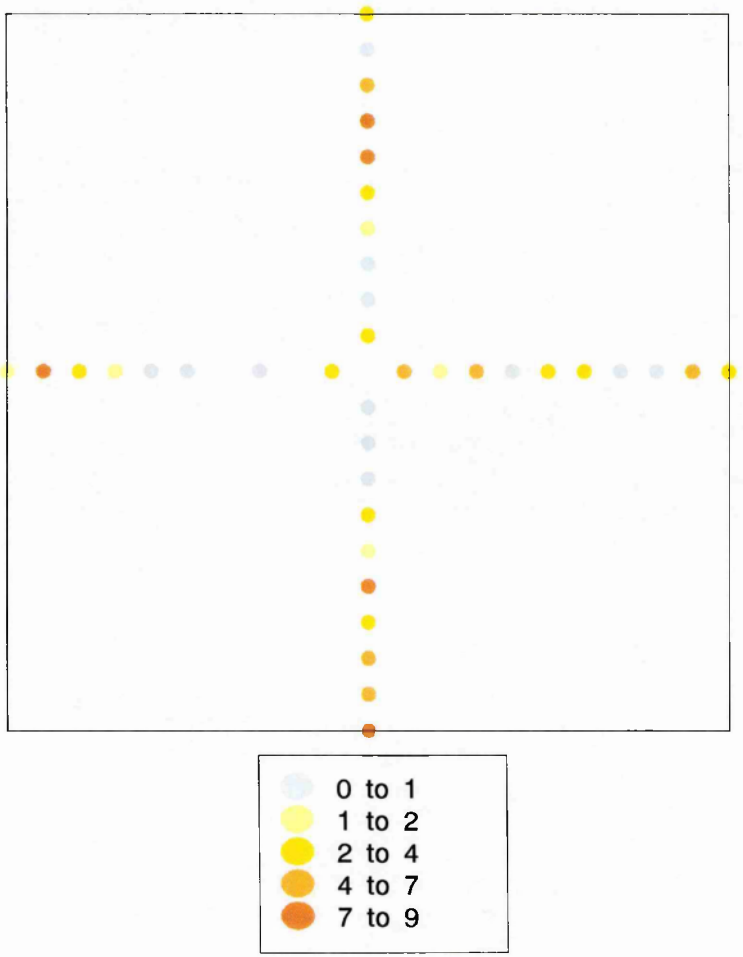


Table 8.3 Correlation matrix for daily densities of *M. aeneus* at three altitudes, temporal factors and daily meteorological values (n=348). Values are Spearman's rank correlations coefficient (r_s), with significance levels based on the Student's t approximation.

Density 12m	**0.575 *****									
Density 200m	**0.299 *****	**0.293 *****								
Week	**0.166 ****	**0.062**	**0.357 *****							
Mean temp	**0.231 *****	**0.187 *****	**0.458 *****	0.853 ***						
Max temp	**0.291 *****	**0.247 *****	**0.551 *****	0.812 ***	0.965 ***					
Min temp	**0.124 ***	**0.072**	**0.280 *****	0.815 ***	0.916 ***	0.811 ***				
Radiation	**0.268 *****	**0.287 *****	**0.400 *****	0.330 ***	0.448 ***	0.574 ***	0.193 ***			
Rainfall	**-0.106 ***	**-0.135 ***	**-0.390 *****	-0.231 ***	-0.308 ***	-0.368 ***	-0.141 **	-0.421 ***		
% Relative Humidity	**0.132 ***	**0.011**	**-0.141 ****	0.219 ***	0.104	0.021	0.239 ***	-0.418 ***	0.369 ***	
Wind speed	**-0.347 *****	**-0.225 *****	**-0.733 *****	-0.550 ***	-0.528 ***	-0.578 ***	-0.390 ***	-0.298 ***	0.299 ***	-0.139 **
	Density 1.5m	**Density 12m**	**Density 200m**	Week	Mean temp	Max temp	Min temp	Radiation	Rainfall	% Relative humidity

* Significance at the 95% confidence level. ** Significance at the 99% confidence level. *** Significance at the 99.9% confidence level.

Table 8.4 Correlation matrix for period of winter rape immigration (n=40). Mean number of *M. aeneus* and proportion of the crop in flower correlated with data for the previous week's meteorological values and insect density at three altitudes. Values are Spearman's rank correlation coefficients (r_s), with significance levels based on the Student's t approximation.

Week	**0.806 *****											
Proportion flowering	**0.809 *****	0.858 ***										
Density 1.5 m	**0.638 *****	0.566 ***	0.462 **									
Density 12 m	**0.563 *****	0.409 **	0.295	0.721 ***								
Density 200m	**0.604 *****	0.554 ***	0.443 **	0.784 ***	0.757 ***							
Mean temp	**0.618 *****	0.602 ***	0.453 **	0.796 ***	0.623 ***	0.756 ***						
Max temp	**0.694 *****	0.537 ***	0.417 **	0.712 ***	0.771 ***	0.726 ***	0.776 ***					
Min temp	**0.462 ****	0.514 **	0.310	0.193	0.244	0.381 *	0.596 ***	0.516 **				
Radiation	**0.826 *****	0.835 ***	0.816 ***	0.703 ***	0.560 ***	0.685 ***	0.662 ***	0.640 ***	0.295			
Rainfall	**-0.355 ***	-0.287	-0.421 **	-0.281	-0.173	-0.138	-0.161	-0.259	0.327 *	-0.589 ***		
% Relative humidity	**-0.710 *****	-0.696 ***	-0.692 ***	-0.616 ***	-0.516 **	-0.481 **	-0.623 ***	-0.609 ***	-0.151	-0.892 ***	0.748 ***	
Wind speed	**-0.132**	-0.244	-0.193	-0.357 *	-0.117	-0.521 **	-0.083	-0.090	0.145	-0.259	0.048	-0.035
	Mean *M. aeneus*	Week	Proportion flowering	Density 1.5 m	Density 12 m	Density 200 m	Mean temp	Max temp	Min temp	Radiation	Rainfall	% Relative Humidity

Significance at the 95% confidence level. ** Significance at the 99% confidence level. *** Significance at the 99.9% confidence level.

Table 8.5 Correlation matrix for period of winter rape emigration (n=40). Mean number of *M. aeneus* and proportion of the crop in flower correlated meteorological values and insect density at three altitudes from the same week. Values are Spearman's rank correlation coefficients (r_s), with significance levels based on the Student's t approximation.

Week	**-0.312 ***											
Proportion flowering	**0.510 ****	-0.742 ***										
Density 1.5 m	**0.174**	-0.078	0.052									
Density 12 m	**0.103**	0.172	-0.145	0.640 ***								
Density 200m	**0.475 ****	0.076	0.268	0.419 **	0.385 *							
Mean temp	**-0.061**	0.739 ***	-0.461 **	0.168	0.601 ***	0.314 *						
Max temp	**0.058**	0.542 ***	-0.386 *	0.377 *	0.701 ***	0.368 *	0.795 ***					
Min temp	**-0.172**	0.546 ***	-0.446 **	0.139	0.261	-0.104	0.686 ***	0.326 *				
Radiation	**-0.016**	0.242	-0.082	-0.002	0.415 **	0.258	0.543 ***	0.730 ***	0.026			
Rainfall	**-0.172**	-0.384 *	0.125	-0.247	-0.384 *	-0.588 ***	-0.626 ***	-0.640 ***	-0.138	-0.611 ***		
% Relative humidity	**-0.407 ****	-0.031	-0.170	0.311	-0.029	-0.481 **	-0.276	-0.358 *	0.226	0.549 ***	0.579 ***	
Wind speed	**-0.007**	-0.523 **	0.270	-0.225	-0.235	-0.658 ***	-0.380 *	-0.235	-0.076	0.075	0.420 **	0.111
	Mean *M. aeneus*	Week	Proportion flowering	Density 1.5 m	Density 12 m	Density 200 m	Mean temp	Max temp	Min temp	Radiation	Rainfall	% Relative Humidity

* Significance at the 95% confidence level. ** Significance at the 99% confidence level. *** Significance at the 99.9% confidence level.

Table 8.6 Correlation matrix for period of spring rape immigration (n=9). Mean number of *M. aeneus* and proportion of the crop in flower correlated with data for the previous week's meteorological values and insect density at three altitudes. Values are Spearman's rank correlation coefficients (r_s), with significance levels based on the Student's t approximation.

Week	**0.788 ***											
Proportion flowering	**0.792 ***	0.459										
Density 1.5 m	**0.669 ***	0.813 **	0.154									
Density 12 m	**0.681 ***	0.782 *	0.189	0.848 **								
Density 200m	**-0.150**	-0.288	-0.145	0.151	-0.092							
Mean temp	**0.200**	0.102	0.323	0.109	-0.193	0.183						
Max temp	**-0.033**	-0.280	0.119	-0.008	-0.235	0.567	0.783 *					
Min temp	**0.617**	0.509	0.366	0.519	0.336	-0.117	0.700 *	0.333				
Radiation	**-0.266**	-0.254	0.187	-0.318	-0.546	0.183	0.233	0.350	-0.383			
Rainfall	**0.067**	0.195	-0.272	0.126	0.345	-0.383	-0.550	-0.750 *	0.033	-0.783 *		
% Relative humidity	**0.117**	0.525	-0.400	0.636	0.571	-0.283	-0.333	-0.467	0.117	-0.317	0.367	
Wind speed	**0.300**	0.288	0.502	-0.259	0.059	-0.833 **	-0.217	-0.533	-0.017	-0.067	0.200	-0.050
	Mean *M. aeneus*	Week	Proportion flowering	Density 1.5 m	Density 12 m	Density 200 m	Mean temp	Max temp	Min temp	Radiation	Rainfall	% Relative Humidity

Significance at the 95% confidence level. ** Significance at the 99% confidence level. *** Significance at the 99.9% confidence level.

Table 8.7 Correlation matrix for period of spring rape emigration (n=11). Mean number of *M. aeneus* and proportion of the crop in flower correlated meteorological values and insect density at three altitudes from the same week. Values are Spearman's rank correlation coefficients (r_s), with significance levels based on the Student's t approximation.

Week	**-0.673 ***											
Proportion flowering	**0.501**	-0.107										
Density 1.5 m	**0.741 ****	-0.694 *	0.148									
Density 12 m	**0.583**	-0.758 **	0.327	0.679 *								
Density 200m	**-0.401**	0.476	-0.078	-0.243	-0.142							
Mean temp	**-0.519**	0.374	0.000	-0.509	0.059	0.653 *						
Max temp	**-0.402**	0.370	0.095	-0.126	0.092	0.691 *	0.769 **					
Min temp	**-0.574**	0.508	-0.147	-0.587	-0.169	0.717 *	0.872 ***	0.622 *				
Radiation	**0.246**	-0.434	0.304	0.216	0.790 **	0.169	0.543	0.330	0.333			
Rainfall	**0.531**	-0.614 *	-0.143	0.870 ***	0.457	-0.151	-0.443	0.000	-0.518	-0.014		
% Relative humidity	**0.219**	0.236	0.060	0.358	-0.078	-0.123	-0.443	-0.128	-0.516	-0.416	0.264	
Wind speed	**0.246**	-0.231	0.060	-0.055	-0.233	-0.826 **	-0.689 *	-0.865 **	-0.653 *	-0.370	-0.165	0.078
	Mean *M.* *aeneus*	Week	Proportion flowering	Density 1.5 m	Density 12 m	Density 200 m	Mean temp	Max temp	Min temp	Radiation	Rainfall	% Relative Humidity

* Significance at the 95% confidence level. ** Significance at the 99% confidence level. *** Significance at the 99.9% confidence level.

8.5 DISCUSSION

The work in this chapter has shown for the first time, an altitudinal profile of *M. aeneus* throughout their active season. It has provided evidence that this species uses high altitude (up to 200 m) as well as low altitude flights. It has also confirmed that there is a strong bias for flight during the day, which might have resulted from a temperature threshold requirement for flight and/or a strong reliance on visual cues during flight at altitude that are unavailable at night.

Flight in *M. aeneus* is most common at low altitude, indicating high levels of population redistribution on a local scale. Such dispersal enables the population to relocate to the ephemeral, but highly concentrated, resource of oilseed rape, their main source of food and oviposition sites (Macdonald & Smith, 1990). Emergence of overwintered adults in March - early April is followed by flights at 12 m. This is evidence for medium range dispersal movements from the overwintering site to feeding sources. Mating occurs on crucifers from mid-May onwards (Williams & Free, 1978), so dispersal from overwintering sites also ensures mate location and increases population heterogeneity (Macdonald & Smith, 1990). The emergence seems to be spread over several weeks that are characterised by warmer weather and low rainfall. However, after emergence, these meteorological factors have less impact on the timings of flights throughout the rest of the season.

During the winter rape colonisation period, there is still a considerable level of flight activity, which could be due to continuing emergence from overwintering or population redistribution to localised areas of resource availability. Following this period, there is an interesting lull in flight activity that coincides with a drop in the number of beetles on the crops. As identified in Section 8.4.1, this occurs in the time between winter rape flowering and spring rape flowering, during which time there is no detectable movement of beetles to other food sources. This might indicate that a large proportion of the overwintered adults die at the end of the winter rape flowering and it is mainly the new generation adults that colonise the spring rape. However, it is thought that the new generation does not reproduce in their first year (Williams & Free, 1978), yet the spring rape crops can be severely damaged by ovipositing females, therefore some reproductive females must move from the winter to the spring crops.

Spring rape immigration is very rapid and the population of *M. aeneus* reaches its peak in mid-July, with the arrival of new generation adults. The use of low altitude flights at this stage is the most efficient way of quickly locating the nearest resource that is still in abundance at this time. Spring rape emigration at the end of the active season is followed by migratory flights at high altitude. Migration prior to overwintering increases genetic variation in the population in the following year and increases the range over which they will emerge.

Following immigration to rape fields, no spatial pattern in the distribution of beetles could be found. There was no evidence for spatial pattern or an edge distribution despite previous suggestions that there are higher numbers of pollen beetles at the crop edge due to movement from flowering plants in crop verges (Free & Williams, 1978). The lack of the edge effect might be due to the additional immigration of insects from the air arriving randomly in the crop, however, there is also the possibility that these insects redistribute quickly on arrival and the initial spatial pattern could have been missed in this study.

The work in this chapter has resulted in a description of the flight patterns of *M. aeneus,* which will be of great importance in implementing the push-pull strategy most effectively. The push-pull strategy is likely to be most effective at controlling pests during immigration to oilseed rape crops. As the experiments in Chapter 6 showed, the use of non-host plant odour is only effective at repelling *M. aeneus* during immigration: once the insects are on the crop plants, non-host odour ceases to be effective. Important considerations include the fact that the immigration pattern is generally linked to the crop phenology and the arrivals have not just moved from nearby flowering plants, but have potentially flown from long distances at a range of altitudes to reach the field.

The novel combination of techniques used in this chapter has provided a method for long-term monitoring of the population movements of this pest, and the modelling of several years worth of data could potentially yield specific predictors of immigration to rape crops.

CHAPTER 9. GENERAL DISCUSSION

Meligethes aeneus is an important pest of oilseed rape throughout Europe. At present, methods used for their control are environmentally harmful and, as such, there is a need for control strategies that avoid the use of pesticides. This thesis followed a logical series of experiments in order to identify plant-derived odours that are repellent to *M. aeneus*. The experiments were also designed to develop an understanding of the olfactory aspects of insect host-location behaviour. Olfaction is an important part of host-plant recognition in many phytophagous insects, including *M. aeneus* (Smart *et al.*, 1995; Blight & Smart, 1999; Smart & Blight, 2000), therefore the potential exists for disruption of this behaviour using repellent non-host plant odours. These two strands to the research have provided a strong scientific basis for the development of a repellent element within a push-pull pest management strategy for oilseed rape.

The research has followed a logical progression of behavioural experiments at expanding spatial and temporal scales. The spatial scale has progressed from a refined scale of a few centimetres in laboratory experiments (Chapters 3 & 4), through a few metres in the semi-field cages (Chapter 6), to a field scale of several meters (Chapter 7) and landscape scale of hundreds of metres (Chapter 8). The temporal scale has progressed from a refined scale of 5-30 minute tests in the laboratory (Chapters 3 & 4), to 1-24 hour experiments in cages (Chapter 6), through daily counts in the field (Chapter 7) and weekly counts over the season (Chapter 8).

9.1 IDENTIFICATION OF REPELLENT NON-HOST PLANT ODOURS TO *MELIGETHES AENEUS*

The approach taken through this thesis followed the sequence of experimental stages appropriate for investigating insect responses to semiochemicals (Poppy, 1991). Namely, behavioural observations, chemical extraction, electrophysiological investigation, chemical identification/synthesis, behavioural bioassays and finally, field trials. This sequence was expanded to include an extra stage, semi-field trials, between behavioural bioassays and full field trials. The semi-field stage proved to be the most valuable for detailed investigation into the responses of the beetle in flight, which was missing from the other stages. Evidently this process would need to be adapted for other species of insects and

crop systems, but, the addition of the semi-field stage could, in many cases, prove to be a beneficial intermediate between laboratory and field scale investigations.

The observation that essential oils of non-host plants reduced host-plant colonisation by *M. aeneus* in choice laboratory bioassays was the initial stage in this investigation (Chapter 3). Lavender essential oil was selected at this preliminary stage to provide a model non-host plant odour that could be used to develop the specific techniques required for testing the behavioural responses of *M. aeneus*. This also provided a strong positive standard against which other potential repellent odours could be tested. The botanical survey of wild flower hosts also provided a candidate non-host plant that was investigated for repellent volatiles (Appendix 1). Chemical extraction from the non-host, *Chamomilla suaveolens*, was achieved by water distillation of vegetative and flowering parts of the plants. Essential oils were used throughout this study as they provide a substantial volume of product that volatilises easily to present a consistent non-host odour cue in behavioural testing.

The use of repellency values (Scheffler & Dombrowski, 1993) (Chapter 3) provided a concise way to summarise the results from the laboratory bioassays and thereby enabled comparisons of several odours and concentrations. Overall, lavender essential oil proved to be the most effective repellent, and so it was used throughout the stages of research and could potentially provide an end-product for use in pest control (section 9.3).

As noted by Dethier (1947), repellents, in many cases, need to be strong enough to compete with an attractant. Therefore, for a repellent to be effective in pest control, it must mask the host plant attractant volatiles in addition to being repellent *per se*. The 4-arm olfactometer provided evidence that the non-host odour from lavender essential oil was sufficiently active to repel *M. aeneus* even in the absence of attractant plant volatiles in the control (Chapter 4; experiment 3).

Electrophysiology coupled with GC was used to establish the identity of the behaviourally active chemicals in lavender essential oil (Chapter 5). Chemical identifications were provided for these active chemicals by means of GC-MS, and these were confirmed using GC peak enhancements. However, further behavioural bioassays were required to confirm the behavioural response of *M. aeneus* to these chemicals and the 4-arm olfactometer was used to test them individually. Linalool, linalyl acetate and 3-octanone were shown to have

repellent effects on *M. aeneus*. In the future, it would be of interest to test behavioural responses to varying ratios of these chemicals to potentially improve on the repellent effect achieved with lavender oil.

The repellency of non-host plant odours to *M. aeneus* was also established at both the semi-field (Chapter 6) and field scale (Chapter 7). Overall, this methodological sequence has proved to be appropriate for investigation of semiochemical repellency. The addition of the semi-field scale trials enabled the practical issues to be resolved in terms of the application of the repellent at larger scales, and under field conditions, before embarking on full field trials. The change from odour release from filter paper in the laboratory to slow-release sachets in the field provided the solution to the odour presentation. However, it still remains difficult to accurately calculate the release rate from such slow-release sources and even more difficult to evaluate the active-space detection range of the insects.

9.2 UNDERSTANDING OF THE OLFACTORY ASPECTS OF HOST LOCATION BEHAVIOUR IN *MELIGETHES AENEUS*

At the outset of this investigation, host location flights of *M. aeneus* were conceptualised as a four-stage process (Figure 9.1).

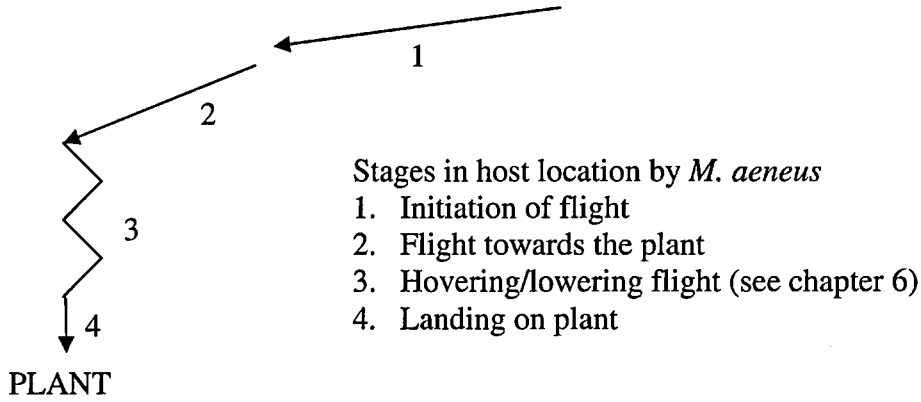


Figure 9.1 Schematic diagram of host location flights by *M. aeneus*

Having identified lavender essential oil as a strong repellent cue, it was subsequently used as a tool to probe these different stages in host location to identify points at which olfactory cues are of high relevance.

The initiation of flight was investigated in Chapter 8 by the use of a novel combination of sampling methodologies to provide a daily altitudinal profile of this species during its active season. By linking this with the colonisation patterns of oilseed rape fields, the population distribution and movements were characterised. It was shown that *M. aeneus* flies at a range of altitudes and up to at least 200m, but predominantly utilises low-level flights. The seasonal patterns of flights indicate that the movements are most strongly correlated with the phenology of their host plants. Therefore, stage 1 of host location is initiated by detection of the appropriate host-plant cues. However, this study did not investigate the mechanisms by which *M. aeneus* is able to detect the availability of host plants over long distances. Evidence suggests that olfaction is important in this process as individuals have been shown to locate oilseed rape fields over a distance of at least 13.5 km using a radioactive tracer (Tamir *et al.*, 1967) and also to use upwind anemotaxis to locate host plants (Evans & Allen-Williams, 1994).

Findings from Chapter 8 suggest that meteorological factors only influence flight behaviour at the beginning of the season when overwintered adults are making their first flights after diapause. However, long-term collection of data from this combination of methodologies would provide a much more conclusive representation of the interaction between flight patterns and climatic conditions. Modelling of these long-term data could identify specific conditions that are prerequisites for flight and thereby enable predictions of crop invasions.

The olfactory and/or physical cues that insects receive, and to which they respond, when flying at high altitudes remain unknown. It is probable that colour vision is important, and insects are known to respond to a pattern of stimuli moving across the visual field, known as the optomotor reaction (Miller & Roelofs, 1978). But since the insects are being carried by the wind at quite high speed, they will be travelling too fast to be able to land upon a target, even as large as an oilseed rape field. Therefore, flight at such heights is restricted to migratory movements (mass movements of the population to another location) at the end of the season (Chapter 8). Searching for suitable habitats and host plants (Stage 2) is only feasible during flight at lower altitudes as the flight path and speed can be altered in response to visual or olfactory cues detected. Additionally, odour filaments are very dispersed at high altitudes, but potentially, detection of a burst of attractant odour might provide the cue to switch to low altitude, searching flight movements.

The fact that most flights occur during the day (Chapter 8) (Lewis & Taylor, 1965) leads to the inference that visual cues are available to most of the beetles during their host-location flights. It is known that *M. aeneus* is highly sensitive to colour and, like many other flower-visiting insects, they are strongly attracted to the colour yellow (Blight & Smart, 1999). Both visual and odour cues have been shown to affect orientation of *M. aeneus* to traps and there is also a significant interaction between these two cues (Blight & Smart, 1999). The use of olfactory cues by *M. aeneus* during orientation towards visually similar host plants was shown in Chapter 6, Experiment 1, as a significant reduction in host plant colonisation occurred in the presence of non-host plant odour.

The behaviour of hovering and lowering on to the plant prior to landing was observed in the field and confirmed as Stage 3 in the host location process (Figure 9.1). This was investigated as the final mechanism for gaining olfactory information prior to landing. Behavioural observations (Chapter 6, Experiment 4) found that non-host plant odour increased the proportion of hovering flights compared to direct landings and led to a higher number of flight path alterations and aborted landings, which together reduced colonisation of the treated plant. The behaviour of the beetle after it has aborted a landing, due to encountering the repellent odour, will be vital to the success of the push-pull strategy and is discussed further in Section 9.3. This behaviour was the final point in the host location process when the non-host plant odour was repellent. After landing (Stage 4), there was no discernible repellent effect of the lavender odour on the behaviour of *M. aeneus* (Chapter 6, Experiment 2). This was corroborated by the similar numbers of beetles seen leaving treated and untreated plants after landing (Chapter 6, Experiment 4) i.e. there was no evidence of increased rejection of the treated plants after landing.

In summary, this thesis has characterised the seasonal flight movements of *M. aeneus* and investigated the flight behaviour of individuals during host-plant location. The importance of olfaction within this behaviour has been established prior to landing, thereby questioning the theory that it is solely dominated by visual cues (Finch & Collier, 2000). But it appears that other factors govern subsequent acceptance behaviour, as non-host plant odour ceased to be repellent.

9.3 DEVELOPMENT OF A PUSH-PULL STRATEGY OF PEST MANAGEMENT IN OILSEED RAPE

Essential oils and their constituents possess varying degrees of pest-controlling properties, including potent sources of pesticides (Dethier, 1947). They can provide safe (i.e. low mammalian toxicity (Isman, 2000)), biodegradable and highly selective pesticides (Singh & Upadhyay, 1993). However, this should not be confused with the additional repellent effect many essential oils have on insects (Sarac & Tunc, 1995). Essential oils have been shown to be repellent to insect pests of stored grains and their products in laboratory and field-based trials (Chander *et al.*, 2000). It is their repellent effect that has been investigated in this thesis and lavender essential oil has proved to be highly repellent to *M. aeneus.*

This thesis has expanded the current understanding of flight behaviour of *M. aeneus* and its response to repellent odours. The stages in host location when olfaction is of importance present the opportunity for the manipulatory aspects of the push-pull strategy to be effective. In theory, repellent odours, such as non-host plant odour can be applied to the crop to deter pest insects. This has been shown to be effective in small plots of oilseed rape during the critical green-bud stage (Chapter 7), although, larger trials are still required to test its effectiveness at the full field scale.

The close proximity of a trap crop, such as turnip rape (Buechi, 1990; Cook *et al.*, 2002b), would increase the benefit of the repellent effects. Not only would fewer insects reach the crop (due to arrestment at the trap crop, where treated with a pesticide or pathogen), but also the displaced insects would have an alternative cue to orientate towards after rejection of the repellent-treated areas. The plasticity shown by *M. aeneus* in field trials (Chapter 7) indicates that these insects are utilising the strongest or most appropriate cue at any given time (Bernays, 1999). Therefore, the unexpected increase in colonisation of the repellent-treated plots may have been avoided if a highly attractive trap crop that flowered at a different time to the main crop was incorporated into the experiment to provide a strong attractant signal to the displaced insects.

As discussed in sections 9.1 & 9.2, the repellent would be most effective if applied to the crop before pest colonisation and caused the insects to move to another location (Potting *et al.*, 2002). Repellent or antifeedant effects of treatment may prove advantageous over the

knockdown effect of conventional pesticides as they have a longer lasting effect (Passerini & Hill, 1993). Monitoring of populations in the fields provides the farmer with an indication of the current infestation levels, but since mass colonisation can often occur suddenly (Chapter 8), this method may miss the short window of opportunity for application of the repellent treatment. As already suggested, modelling of long-term data of population movements could provide predictors of pest outbreaks, giving advance warnings.

The non-host plant odour could be incorporated into the management of the crop in several ways. Firstly, non-host plants could be grown alongside the crop plants i.e. intercropping. This is effective on a small scale (Onesimus *et al.*, 1998; Hooks & Johnson, 2001), however it is highly labour intensive to sow and harvest a crop interplanted with other plants and for these reaons, the practicalities of intercropping are problematic for large-scale cultivation of oilseed rape in the UK. Planting repellent plants as a border around the outside of the field provides a manageable alternative, but predictions from a simulated model of insect movement (Roel Potting, unpublished data) indicate that this deployment of repellent plants would have the effect of concentrating the insects in the centre of the crop at the maximal distance from the repellent border. The border design works well for attractant trap crops that arrest colonising insects before they reach the crop, whereas repellents are more effective when applied within the crop as the volatiles are spread more evenly across the area.

A limitation on the interpretation of the results from this thesis is that the volatiles emanating from whole lavender plants in natural situations have not been identified or tested for repellency to *M. aeneus*. A similar study using a different species complex, found repellent responses to ovipositing *Plutella xylostella* L. of essential oils and herb extracts (Dover, 1985). But while intercropping brassicas with these herbs did reduce oviposition, the effect was shown to be due to their barrier effect restricting access to the host plant, and not due to the herbs extending their natural defence to the brassicas (Dover, 1986). However, all biological systems vary and such results cannot always be extrapolated between species. The results from this thesis may be more encouraging as they have provided evidence of a repellent response of *M. aeneus* to lavender volatiles on a field scale. Additionally, both lavender oil (Sellar, 1992) and the plant (*Lavandula angustifolia* (Wiesenfeld, 1997)) are known to have insect repellent and antifeedant

(Koschier *et al.*, 2002) properties. Lavender is recommended to gardeners as an insect repellent, companion plant (Boldweb.com, 2003; Pioneerthinking.com, 2003), therefore lavender intercrops could potentially provide a barrier and repellent action to insect pests.

The alternative to intercropping is to apply the repellent odour as a formulated spray or from slow-release point sources within the crop. It would be of interest to test the potency of lavender essential oil applied as a repellent spray against pests of oilseed rape, however it would first need to be tested for any side effects (Trumble, 2002) and formulating to prevent it from volatilising too quickly. The only example of spraying a repellent on field crops is the use of formulated honeybee alarm pheromone (2-heptanone and isopentyl acetate) to repel bees from crops before insecticide application (Free *et al.*, 1985; Rieth *et al.*, 1986). This approach was effective in reducing the number of foraging bees on oilseed rape and other flowering crops, however, the volatility of the pheromones led to only a transient repellent effect. Future research needs to be aimed at development of technologies that prolong the release of semiochemicals for field application.

Therefore, repellents have a potential central role to play within the proposed push-pull pest management strategy for oilseed rape in terms of manipulating both pests and beneficial insects. This thesis has shown the strong influence of olfactory cues during host-plant location of the pest insect *Meligethes aeneus* and the repellent effect of non-host plant volatiles.

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APPENDIX 1. SURVEY OF WILD FLOWERS FOR NON-HOST PLANTS OF *MELIGETHES AENEUS*

Botanical survey

The natural host range of pollen beetles on wild flowers was surveyed to give an indication of plants, which may potentially have repellent odours. Throughout the summer of 2001, the flowering wild plants on field margins of oilseed rape fields on Rothamsted farm, Harpenden, Herts were surveyed weekly. All flowering plants were identified using the key in Collins Wild Flower Guide (Fitter *et al.*, 1993). The presence/absence of feeding pollen beetles was also recorded. The species of pollen beetle was impossible to identify in the field; therefore, the absence of *all* species of pollen beetle was taken to be a good indication of the plant being a non-host.

The botanical survey was conducted weekly from 12/3/01 to 20/8/01 and 52 species of flowering plant were surveyed for the presence or absence of feeding pollen beetles. Fifty-two plant species were identified belonging to 24 families. Pollen beetles were observed feeding on 32 species, and 20 were never observed to have feeding pollen beetles (Table A1). Throughout March, flowering plants were recorded but without any sighting of pollen beetles. However, in early April the first pollen beetles emerged from winter diapause and from that point onwards were frequently found on their flowering host plants until the end of August.

All the wild crucifers were observed to be colonised by pollen beetles, along with most of the Rosaceae (except for agrimony). The Polygonaceae (docks, sorrel & buckwheat) were all non-hosts for pollen beetles. Apart from these examples, there was a mixture of host and non-host plants in the rest of the plant families. In general the host plant flowers were yellow or white and the non-host flowers were blue, red or purple, however there were examples in both classes where this trend was reversed. Also, non-host plants where characterised by having very small flowers e.g. cleavers, chickweeds etc.

From the non-host plants identified from the botanical survey, pineapple mayweed, *Chamomilla suaveolens* was selected to test in bioassays for its potential as a repellent to pollen beetles. The justification for this was that its flowers were the usually attractive

white-yellow colour and a co-existing, related species, scentless mayweed *Matricaria perforata*, was heavily colonised by pollen beetles whereas none were seen on *C. suaveolens* throughout the season. Additionally, *C. suaveolens* has a strong scent that may be acting as a repellent. Therefore, the essential oil of this plant was extracted.

***Chamomilla suaveolens* essential oil extraction**

Ten 5" pots were densely sown with *C. suaveolens* seeds (Herbiseed, Wokingham). The plants were grown in the glasshouse for three months. Once flowering, all the vegetation (leaves, stems and flowers) were harvested from the plants in all the pots. The plant material was placed in a 20-litre round bottomed flask with a flanged top and 2l of boiling water was added. To the Dean and Stark receiver, 6ml of isooctane and 6ml of ethyl acetate were added. Two condensers were added to the Dean and Stark receiver and the small mantle was switched on to maximum. Once the steam and oils generated from the plant material started to condense and drip through the organic solvents in the Dean and Stark receiver, the mantle was turned down to leave the water simmering. The plant material was left to steam distil for 7 hours. After this time, the apparatus was allowed to cool slightly and once the steam had stopped condensing, the organic and aqueous layers in the Dean and Stark receiver were transferred to a glass vial. The water was drained from the solvent layer using a 50ml separating funnel. The solvent layer containing the plant essential oil was poured into a beaker. The water was returned to the separating funnel and was rinsed three times with 5ml ether. All the organic layers were combined and dried over magnesium sulphate. The magnesium sulphate was filtered from the solution through filter paper - the remaining $MgSO_4$ was rinsed twice with 5ml ether. This dried solution was then evaporated on the rotary evaporator. The sample was pipetted from the flask into a 4ml vial and the round-bottomed flask was rinsed with a small amount of ether three times and this was added to the sample in the vial. Nitrogen at room temperature was blown over the sample in the vial to evaporate the remaining ether. The pure essential oil was weighed, diluted in acetone and stored at -20°C.

245g of plant material was obtained from the potted *C. suaveolens*. The water distillation of the plant material yielded 49.14mg of oil, only 0.02% of the weight of the starting material. This was diluted to 10mg/ml in acetone and used in olfactometer tests in chapter 4.

Table A1. Botanical survey of wild flowers and pollen beetle incidence

	12.3.01	14.3.01	19.3.01	26.3.01	2.4.01	11.4.01	17.4.01	30.4.01	8.5.01
daffodils					1		1		
common chickweed									
field speedwell							1	1	1
groundsel									
red dead nettle									
shepherd's purse					1	1	1	1	1
dandylion					1	1	1	1	1
dogs mercury					1		1	1	1
sweet violet									
lesser celendine					1		1	1	1
white bittercress							1	1	1
hazel catkins									
pineapple mayweed									
bluebells									1
ragwort					1				
white campion					1			1	1
ground ivy									
blackthorn								1	
cherry								1	
field pansy									
yellow deadnettle									1
white deadnettle								1	1
candytuft								1	
forget me not									
hawthorn									
buttercup									
cow parsley									
cleavers									
bladder campion									
scarlet pimpernel									
stinging nettle									
sea kale									
vetch									
sow thistle									
honeysuckle									
dog rose									
cut-leaved cranesbill									
buckwheat									
belladonna									
poppies									
elderflower									
purple thistle									
bramble									
mayweed									
field bindweed									
pink thistle									
creeping thistle									
common sorrel									
docks									
old man beard									
yarrow									
agrimony									
total flowering species with pollen beetles	0	0	0	0	7	2	7	11	10

Green boxes indicate flowering plants sampled on that date, the number 1 in the box represents the presence of pollen beetles and the plant names highlighted in blue in the final column represent plants without a record of pollen beetle incidence.

Table A1. Botanical survey of wild flowers and pollen beetle incidence

	14.5.01	21.5.01	31.5.01	4.6.01	11.6.01	18.6.01	25.6.01	2.7.01	9.7.01	16.7.01
daffodils										
common chickweed										
field speedwell		1								
groundsel										
red dead nettle										
shepherd's purse					1					
dandylion		1	1	1	1					1
dogs mercury										
sweet violet										
lesser celendine										
white bittercress										
hazel catkins										
pineapple mayweed										
bluebells		1								
ragwort										
white campion		1								
ground ivy										
blackthorn										
cherry										
field pansy										
yellow deadnettle		1	1							
white deadnettle		1								
candytuft										
forget me not										
hawthorn			1							
buttercup			1		1		1	1	1	1
cow parsley		1	1	1				1	1	1
cleavers										
bladder campion										
scarlet pimpernel										
stinging nettle			1	1	1	1	1	1		
sea kale			1	1	1					
vetch									1	
sow thistle								1	1	1
honeysuckle									1	1
dog rose							1			
cut-leaved cranesbill										
buckwheat										
belladonna										
poppies								1	1	1
elderflower										
purple thistle										1
bramble								1	1	1
mayweed								1	1	1
field bindweed								1	1	1
pink thistle								1		
creeping thistle										
common sorrel										
docks										
old man beard										
yarrow										
agrimony										
total flowering species with pollen beetles	0	7	7	4	5	1	3	9	9	10

Green boxes indicate flowering plants sampled on that date, the number 1 in the box represents the presence of pollen beetles and the plant names highlighted in blue in the final column represent plants without a record of pollen beetle incidence.

Table A1. Botanical survey of wild flowers and pollen beetle incidence

23.7.01	30.7.01	6.8.01	13.8.01	20.8.01	PB incidence		
					2	daffodils	*Narcissus spp.*
					0	common chickweed	*Stellaria media*
					4	field speedwell	*Veronica persica*
					0	groundsel	*Senecio vulgaris*
					0	red dead nettle	*Lamium purpureum*
					6	shepherd's purse	*Capsella bursa-pastoris*
		1	1	1	13	dandylion	*Taraxacum spp.*
					4	dogs mercury	*Mercurialis perennis*
					0	sweet violet	*Viola odorata*
					4	lesser celendine	*Ranunculus ficaria*
					3	white bittercress	*Cardamine spp.*
					0	hazel catkins	*Corylus avellana*
					0	pineapple mayweed	*Chamomilla suaveolens*
					2	bluebells	*Endymion non-scriptus*
					1	ragwort	*Senecio jacobaea*
					4	white campion	*Silene alba*
					0	ground ivy	*Glechoma hederacea*
					1	blackthorn	*Prunus spinosa*
					1	cherry	*Prunus spp.*
					0	field pansy	*Viola arvensis*
					3	yellow archangel	*Lamiastrum galeobdolon*
				1	4	white deadnettle	*Lamium album*
					1	candytuft	*Iberis amara*
					0	forget me not	*Myosotis arvensis*
					1	hawthorn	*Crataegus monogyna*
1	1	1			9	buttercup	*Ranunculus spp.*
1			1		8	cow parsley	*Anthriscus sylvestris*
					0	cleavers	*Galium aparine*
					0	bladder campion	*Silene vulgaris*
					0	scarlet pimpernel	*Anagallis tenella*
					6	stinging nettle	*Urtica dioica*
					3	sea kale	*Crambe maritima*
1		1			3	vetch	*Viscia spp.*
1		1			5	sow thistle	*Sonochus oleraceus*
1					3	honeysuckle	*Lonicera periclymenum*
					1	dog rose	*Rosa canina*
					0	cut-leaved cranesbill	*Geranium dissectum*
					0	buckwheat	*Fagopyrum esculentum*
					0	belladonna	*Atropa bella-donna*
1	1	1	1		7	poppies	*Papaver rhoeas*
					0	elderflower	*Viburnum spp.*
1	1				3	purple thistle	*Cirsium spp.*
1					4	bramble	*Rubus fruticosus*
1	1	1	1	1	8	mayweed	*Matricaria perforata*
1	1	1	1	1	8	field bindweed	*Convolvulus arvensis*
		1	1	1	4	pink thistle	*Cirsium spp.*
					0	creeping thistle	*Cirsium arvense*
					0	common sorrel	*Rumex acetosa*
					0	docks	*Rumex spp.*
	1			1	2	old man beard	*Clematis vitalba*
		1		1	2	yarrow	*Achillea millefolium*
					0	agrimony	*Agrimonia eupatoria*
10	6	9	6	7			

Green boxes indicate flowering plants sampled on that date, the number 1 in the box represents the presence of pollen beetles and the plant names highlighted in blue in the final column represent plants without a record of pollen beetle incidence.

Appendix 2. Map of Rothamsted Experimental Farm